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JOURNAL OF ANATOMY

SPINAL ORIGIN OF THE VENTRAL SUPRAOPTIC DECUSSATION (GUDDEN'S COMMISSURE) IN THE SPIDER MONKEY*

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INTRODUCTION

The origin of the fibres in the ventral supraoptic decussation has been disputed since its discovery by von Gudden (1870). Earlier workers were largely concerned with demonstrating Gudden's commissure as an anatomical entity. Attempts to determine the source and destination of its constituent fibres have been inconclusive, although various structures above the midbrain, especially the medial geniculate body, have been suggested as sites of origin and termination. That a component of Gudden's commissure originates in the spinal cord has been completely unsuspected, apart from a suggestion by Magoun & Ranson (1942) that such fibres might come from levels below the pons.

In studying the ascending fibre systems from the caudal segments of the spinal cord in the spider monkey, secondary degeneration was seen in Gudden's commissure. At first, considered as an artefact, it was subsequently verified in six preparations. The degenerated fibres in Gudden's commissure, together with other degenerations in some of those specimens, were illustrated graphically in one of our earlier communications to this *Journal* (Chang & Ruch, 1947). The existence of this system of fibres alters our conception of the functional significance of Gudden's commissure, which is based on the belief that it serves as an interconnexion between the medial geniculate bodies or inferior colliculi of the two sides.

MATERIAL AND METHODS

Lesions were made aseptically at selected levels of the spinal cord in five spider monkeys (*Ateles*) and one cat. A second operation was performed on one animal in which the caudal segment of the spinal cord had been divided 6 months previously.

Various periods of time (from 14 to 30 days) were allowed for secondary degeneration of the ascending nerve fibres. Under deep Nembutal anaesthesia, the vascular system was perfused with a mixture of equal parts of 3 % magnesium sulphate and 3 % potassium dichromate, without previous washing with saline. The brain and spinal cord were removed immediately, and placed in 10 % neutral formalin. Every precaution was taken to avoid unnecessary handling of the nervous tissue. After 24 hr. the brain and critical segments of the spinal cord were sliced into sections

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about 3 mm. thick, and trimmed. Selected slices were then transferred, without washing, to the chlorate-osmic-formalin mixture of Swank & Davenport (1935).

The brain slices were placed flat on a sheet of blotting-paper in a Stender dish which was closely covered and left in the dark. The mixture was agitated, and the pieces of tissue turned over daily. After 8 days the slices were washed, dehydrated with alcohols and embedded in celloidin in the shortest possible time. Satisfactory results were obtained by completing the whole process from dehydration to mounting in 2 days. Sections were cut 50 μ thick, and mounted in balsam with glass cover slips.

RESULTS

Experiment 1. *Spider monkey series no.* 13*; adult male. Spinal transection at* Ca_1 (1 *Sept.* 1944). *Killed* 1 *Oct.* 1944

Lesion. At the level of the lesion, the nervous tissue was completely destroyed, and replaced by scar tissue. Microscopical examination reveals no intact nerve fibres there.

Histological examination. Immediately above the lesion degenerated fibres cross in the ventral commissure, pass through the grey matter of the ventral horn, and reach the ventrolateral funiculus. A compact group of degenerated fibres, which can be traced throughout the spinal cord and medulla, is present in the periphery of Gowers' ventrolateral fasciculus. In the midbrain, a number of the degenerated fibres are grouped in the dorsal part of the posterior commissure. The rest of them curve ventrolaterally through, or over, the ventral part of the medial geniculate body and join the fibre bundles along the medial border of the optic tract. Fibres also pass through the dorsal aspect of the basis pedunculi. At the anterior end of the thalamus, the course of the degenerated fibres can be followed along the dorso-medial border of both optic tracts (Pl. 1, fig. 1). Those fibres do not intermingle with the fibre bundles of the dorsal supraoptic decussation—pars ventralis (Meynert's commissure)—although they are in close relation to it. On the other hand, the decussating degenerated fibres are conspicuously present in Gudden's commissure. After crossing they travel along the border of the optic tract toward the ventromedial part of the globus pallidus, but none of the fibres actually enters that nucleus. The final destination of these fibres is not determinable, because the serial sections at this level are incomplete.

Experiment 2. *Spider monkey series no.* 17*; one-year-old female. Spinal transection at* Ca_1 (28 *Nov.* 1944). *Killed* 11 *Dec.* 1944

Lesion. Sections at the level of the transection showed that the spinal cord was completely interrupted. Damage to the spinal cord was somewhat greater on the right side than on the left.

Histological examination. Degenerated fibres on both sides of the cord can be traced from Gowers' fasciculus to the midbrain and thalamus. In this specimen, the midbrain and thalamus were cut in a more frontal plane, giving sections different in appearance from those in Experiment 1. Sections cut at the level of the posterior commissure show a few degenerated fibres, emanating from the spinothalamic tract in the dorsolateral tegmental region, which turn laterally and then ventrally through

the medial geniculate body to its lateral border. Traced upward through several sections, these fibres can be followed to the medial border of the optic tract near the lateral geniculate body. Relatively more abundant are the degeneration products in the fibre layer between the medial geniculate body and the lateral geniculate body. Degenerated fibres are seen in the dorsolateral aspect of the basis pedunculi where their orientation suggests their coming from the region of the nucleus peripedunc-ularis and the posteroventral border of the thalamus. In some sections, especially on the right side, degeneration products are seen about the medial region of the nucleus pregeniculatus where they seem to terminate (Pl. 1, fig. 2). In other sections, fibres near the nucleus pregeniculatus shift dorsally towards the ventral nucleus of the thalamus. At still higher levels, dark degeneration granules are arranged in rows along the junctional border of the optic tract and the fibre bundle of Meynert's commissure which is distinguished by its lighter colour in the Marchi preparations. Degeneration in the optic chiasma is confined to Gudden's commissure.

Experiment 3. *Spider monkey series no.* 14*; adult female. Left hemisection at* Ca_1 (8 *Sept.* 1944). *Killed* 26 *Sept.* 1944

Lesion. The hemisection of the cord was complete except for a part of the funiculus sulcomarginalis near the ventral median sulcus. On the right, only the medial margin of the dorsal column was involved.

Histological examination. Ascending degenerated fibres are present on both sides in the fasciculus ventrolateralis superficialis of Gowers, but are more numerous on the operated side throughout the spinal cord. Before reaching the thalamus, some fibres leave the dorsal tegmental region of the midbrain to curve dorsally or ventrally or to pierce the anterior part of the medial geniculate body, and finally join the most medial fascicle of the optic tract. Sections from the anterior mesencephalon clearly show that degenerated fibres sweep over the most dorsal aspect of the pes pedunculi, and go to the medial border of the optic tract where they enter the lateral geniculate body. Many various-sized particles of degeneration material are collected in the tricuspid area formed by the pes pedunculi, medial geniculate body and the lateral geniculate body (Pl. 1, fig. 3). This can be seen on both sides of the brain.

Pl. 1, fig. 4 demonstrates the presence of degenerated fibres in the posterior part of the optic chiasma, where the unaffected dorsal supraoptic decussation can be seen lying between the third ventricle and the degenerated fibres.

Experiment 4. *Spider monkey series no.* 18*; adult female. Unilateral anterolateral chordotomy at* C_3 *on the right* (5 *Jan.* 1945). *Killed* 26 *Jan.* 1945.

Lesion. Although the primary lesion was confined to the anterolateral funiculus of the right side, some fibres in Flechsig's fasciculus also underwent degeneration, which was probably the result of an accidental trauma or of a vascular disorder occasioned by the near-by lesion. But this does not affect the degeneration picture in the midbrain and thalamus. The primary lesion was a triangular area with an obtuse angle directed medially. The side along the ventrolateral periphery was about 2 mm. in length. The dorsal spinocerebellar tract and the pyramidal tract escaped direct involvement in the lesion. The other side of the cord was normal.

Histological examination. In the anterior part of the midbrain degenerated fibres

are seen in the medial lemniscus, and occupy a fairly large area in the dorsolateral tegmentum on the operated side. The corresponding area on the other side is free of degeneration. At the level of the posterior commissure, the degenerated fibres begin to radiate in various directions. Some, which are directed ventrolaterally, pass through the medial geniculate body and the dorsolateral border of the pes pedunculi. Some degeneration product is deposited in a tricuspid area formed by the nucleus peripeduncularis, and the medial and lateral geniculate bodies. On the other side of the same section, the medial geniculate body contains no degenerated fibres. From the dorsolateral aspect of the pes pedunculi the degenerated fibres can be followed along the medial border of the optic tract to the optic chiasma where they cross in Gudden's commissure (Pl. 2, fig. 7). The number of degenerated fibres in Gudden's commissure in this specimen is greater than in any of the other animals studied. The distribution of the degenerated fibres in the optic tracts is symmetrical.

Experiment 5. *Spider monkey series no.* 19*; adult female. First operation: spinal transection at* Ca_1 (13 *July* 1945). *Second operation: left hemisection at* L_1 (10 *Jan.* 1946). *Killed* 30 *Jan.* 1946

Lesions. Histological sections at the site of the first lesion were not made, but the degeneration picture in the segment above the lesions suggests a complete transection. The medial margin of the second lesion is sharp and does not extend to the right side. The hemisection is complete apart from a very narrow strip along the median line.

Histological examination. At the level of the inferior collicus, degenerated fibres stream dorsally parallel, and largely medial, to the lateral lemniscus. They apparently turn dorsally before the medial lemniscus fibres do, since the main body of the latter still remains in the tegmentum near the pons while the majority of the former has already reached the parabigeminal region beneath the inferior colliculus. At the junction of midbrain and thalamus some degenerated fibres leave the main stream of thalamopetal fibres, pass through the nucleus peripeduncularis around the dorsal aspect of the pes pedunculi and proceed to the medial border of the optic tract. There are few of these fibres—the fewest encountered in the five spider monkeys observed. This system of degenerated fibres, coursing within Gudden's commissure, reappears in the optic tract of the opposite side. The position of the fibres in the chiasma is ventral to Meynert's commissure. The small fibre strand of Ganser's commissure stands out beneath the recess of the third ventricle as a separate unit.

ORIGIN, COURSE AND TERMINATION OF THE SPINAL COMPONENT OF GUDDEN'S COMMISSURAL FIBRES

Origin. Degeneration of fibres in the ventral supraoptic commissure was produced by transection, hemisection and anterolateral section of the spinal cord at various levels. It is evident that some of the fibres in Gudden's commissure have their cells of origin in the spinal cord.

The lower limit of the spinal origin is established by the fact that secondary degeneration was present in Gudden's commissure after transection of the spinal cord at the first caudal segment. Cells in the conus of the spinal cord must contribute

some fibres to that commissure which, therefore, carries some of the longest ascending fibres in the central nervous system.

The caudal cord region is not the sole origin of the supraoptic decussational fibres in question, since lesions at higher levels of the spinal cord produce more decussational degeneration than does a spinal transection at the caudal region. Especially, the number of degenerated fibres in Gudden's commissure is greater (though not very much greater) in spider monkey no. 18 (with an anterolateral chordotomy at C_3) than in the animals no. 13 and no. 17 (spinal transection at Ca_1) or no. 14 (spinal hemisection at Ca_1). Thus, additional fibres must be contributed from the segments between C_3 and Ca_1. Furthermore, degenerated fibres in Gudden's commissure are demonstrable following a hemisection at the level of the first lumbar segment of a monkey in which the caudal cord had been transected 6 months previously. The appearance of new degeneration in the decussation proves that the lumbar and sacral segments contribute fibres to the ventral supraoptic decussation. Therefore, it is believed that this system of fibres originates from all levels of the spinal cord, but the contribution from the caudal segments of the spider monkey, which are very highly developed (Chang & Ruch, 1947*a*), seems disproportionately great. It remains unknown which group of cells in the grey matter of the spinal cord gives origin to these fibres.

Unilaterality versus bilaterality. Hemisection of the spinal cord gives rise to some degeneration in the anterolateral funiculus of both sides. However, degenerated fibres traversing the medial geniculate body are present only on the operated side (Pl. 2, fig. 6). The medial geniculate body on the unoperated side is virtually free of degenerated fibres (Pl. 2, fig. 5), despite their fairly large number in the anterolateral column on the unoperated side. It is assumed that the degenerated fibres ascending contralaterally to the lesion are of the same category as the bulk of the fibres ascending on the side of the lesion—namely, spinothalamic, ventral spinocerebellar fibres, etc.—originating at the site of the lesion, or crossing obliquely through the lesion. Since such contralateral fibres do not pass through the contralateral geniculate body, as mentioned above, it follows that similar fibres crossing the spinal cord below the lesion and ascending on the side of the lesion are not responsible for the degeneration in the ipsilateral medial geniculate region. This suggests that fibres ascending from the lesion to the decussation must originate on the same side of the spinal cord as, and below the level of, the lesion.

Course. If the above is correct, axons destined for the supraoptic decussation, after emerging from cells in the grey matter of the spinal cord, turn laterally to the ventrolateral funiculus on the same side, where they intermingle with the fibres of other ascending systems and ascend the cord without much change in course. In the lower medulla, they occupy, along with the others, a region between the inferior olive and the descending trigeminal tract at the ventrolateral periphery of the medulla. After the divergence of the spinobulbar and spinocerebellar components from the composite fibre bundle of Gowers' fasciculus, they proceed farther upwards in company with the spinocollicular and spinothalamic fibres. They cannot be distinguished topographically from the fibres of the other systems throughout their ascending course until the posterior end of the thalamus is reached, where they curve laterally and pass through the dorsal end of the basis pedunculi, the medial

geniculate body, the space between these two structures and the medial border of the optic tract. They cross to the opposite side at the posterior part of the optic chiasma. Immediately before and after the crossing, they remain at the dorsal border of the chiasma and beginning of the optic tract. Ganser's commissure is completely separated from them by a band of fibres probably belonging to Meynert's commissure. Here, in the chiasma, the fibres of Gudden's commissure appear a little more scattered and more wavy than they do in the optic tract.

After crossing, they are grouped in a small bundle along the dorsomedial border of the optic tract on the other side in exactly the same position that they occupied before decussation. The post-decussation fibres come to lie in a triangular area formed by the medial geniculate body, the lateral geniculate body, and the dorsal end of the pes pedunculi, where they disappear.

Termination. The exact termination of the spinal component of the supraoptic decussational fibres is not entirely clear. Repeated study of all our Marchi preparations suggests that two regions constitute the end-stations of these devious fibres: (1) the medial portion of nucleus pregeniculatus, and (2) the region immediately beneath the medial geniculate body. The nucleus pregeniculatus in the spider monkey, as described by Solnitzky & Harman (1943) consists of two portions, a parvicellular lateral portion and a medial portion containing large cells in the centre and small cells in its peripheral zone. Degeneration in the present material is confined to the medial portion of this nucleus.

No fibres can be followed to any other structure of the brain. The present results would seem to exclude the existence of any connexions between these fibres and the medial geniculate body, quadrigeminal bodies, globus pallidus, supraoptic nuclei or any of the hypothalamic cell groups.

DISCUSSION

By experimental degeneration, von Gudden (1870) first demonstrated that supraoptic decussations are not a part of the visual system despite their proximity to the optic chiasma and the optic tract. Since then, additional supraoptic decussations were described by Meynert and Ganser. None of the earlier writers determined the exact origin of these decussating fibres. Von Gudden believed that the fibres in the 'commissura inferior' (ventral supraoptic decussation), which leave the optic tract to pass over the most lateral part of the pes pedunculi, have their origin in the cerebral cortex. However, no such relationship is indicated by decortication experiments in several species—Papez & Rundles (1938), Papez (1938), Magoun & Ranson (1942), and Mettler (1945). That Gudden's commissure connects the medial geniculate bodies and mediates auditory impulses is taught in many text-books of neuroanatomy, and is supported by several recent workers (Rioch, 1931; Bodian, 1940; Kuhlenbeck & Miller, 1942) chiefly from materials prepared with normal myelin sheath stains. On the other hand, recent studies with Marchi's technique by Wollard & Harpman (1939) and Ades (1941) question the relationship of Gudden's commissure to the medial geniculate nuclei. A relationship of Gudden's commissure to the colliculi has been described by Obersteiner (1912) and by Gerebtzoff (1939); the relationship to the supraoptic nuclei noted by Schafer & Symington (1908), Tilney & Riley (1938) and Tsang (1940) is inferential rather than experimental.

A new component of the ventral supraoptic decussation was described by Magoun & Ranson in 1942. They studied the Marchi degeneration produced by well-localized Horsley-Clarke lesions in the midbrain of cats and monkeys. They established that fibres destined for the ventral supraoptic decussation ascend to the anterior end of the midbrain in the lateral part of the tegmentum; pass through the basis pedunculi and along the medial border of the optic tract; and, after crossing, enter the ventrolateral part of the midbrain. Some fibres may terminate in the medial geniculate body, and rostral tectal and pretectal regions, but most seem to end in the hypothalamus. Apart from the suggestion that they may be derived from the lower brain stem—some as far back as the pons—their material did not permit determination of the origin of supraoptic decussational fibres. The possibility of the bulbar and spinal components in the supraoptic commissures has been entirely overlooked apart from a recent study by Glees (1944) who demonstrated that some fibres emerging from the medial lemniscus contribute to the dorsal supraoptic decussation (Ganser's commissure).

In their analysis of the *Ateles* optic tract, Solnitzky & Harman (1943) note that a small bundle of their medial fascicles goes to the medial portion of the nucleus pregeniculatus, and the region medial to the zona incerta. These authors, despite the peculiarity of this separation and termination of this fascicle, considered it a part of the optic system. The present study suggests that the fibres in question are of non-optic origin, and belong to the system of supraoptic commissures. However, all the fibres in the most medial fascicle probably are not alike. Since in none of our experiments was complete degeneration of the fascicle produced, spinal fibres must be only a constituent of it.

Phylogenetic considerations. In our Marchi preparations of spider monkeys with transection, hemisection or anterolateral section of the spinal cord at various levels, degenerated fibres in the supraoptic commissure are invariably traceable to the region between the lateral geniculate body and the subthalamus corresponding to the nucleus pregeniculatus. If the belief of Minkowski (1920) and Le Gros Clark (1932)—that the nucleus pregeniculatus of the primates and man is phylogenetically derived from the pars ventralis of the lateral geniculate body—is correct, it is reasonable to expect fibre degeneration in the pars ventralis of the lateral geniculate body in lower mammals after spinal transection. With this thought in mind, an anterolateral chordotomy was made at the second cervical segment in a cat. The result was disappointing, since no degenerated fibres could be traced above the pons, although they were profuse in Gowers' fasciculus in the spinal cord and medulla. Weaver (1937) was unable to find Gudden's commissure in a cat with all optic fibres degenerated due to the enucleation of the eyes. Beck (1927) also could not trace degenerated fibres beyond the pons after a lesion of a cat's spinal cord.

Papez's work on the chronically hemidecorticate dog gives some information concerning this point. He could trace intact fibres of the ventral supraoptic decussation to the pars ventralis of the lateral geniculate body, the ventral part of the medial geniculate body and the anterior tegmental nucleus. Although it is difficult to determine the polarity of these fibres in Weigert preparations, Papez believed that they originate in these nuclear groups. The fibres traceable to the anterior tegmental nucleus in Papez's material seem to coincide with the predecussational

course of the supraoptic fibres of spinal origin in Gudden's commissure; and those leading to the pars ventralis of the lateral geniculate body, with the post-decussational fibres of Gudden's commissure. With respect to the human brain, Gardner & Cuneo (1945), on the basis of Marchi preparations from a patient who died 21 days after an anterolateral chordotomy at the upper thoracic portion of the cord, briefly described degenerating fibres ascending to the medial geniculate body. They did not describe the further course of those fibres. In the light of the present study, it is quite possible that these were supraoptic decussational fibres of spinal origin.

SUMMARY

A previously undescribed component, of spinal origin, in Gudden's commissure was demonstrated by the Marchi method in five spider monkeys. Degenerated fibres were found in Gudden's commissure after transection, hemisection or anterolateral chordotomy at various levels of the spinal cord. Following spinal lesions some of the ascending degenerated fibres in Gowers' fasciculus can be traced upwards to the anterior end of the midbrain where some of them pass through the dorsal aspect of the basis pedunculi, the medial geniculate body, and the space between these two structures to join the medial border of the optic tract. After crossing in the ventral supraoptic decussation of Gudden, the degenerated fibres turn back along the medial border of the optic tract of the opposite side and finally disappear into the region of the nucleus pregeniculatus and the region beneath the medial geniculate body. None of these fibres was observed terminating in the hypothalamus or the globus pallidus, nor did they appear to traverse or to end in the medial geniculate body of the side opposite to the lesion.

REFERENCES

Ades, H. W. (1941). Connections of the medial geniculate body in the cat. *Arch. Neurol. Psychiat., Chicago*, **45**, 138–144.

Beck, G. M. (1927). The cerebellar terminations of the spino-cerebellar fibers of the lower lumbar and sacral segments of the cat. *Brain*, **50**, 60–98.

Bodian, D. (1940). Studies on the diencephalon of the Virginia opossum. II. The fiber connections in normal and experimental material. *J. comp. Neurol.* **72**, 207–297.

Chang, H. T. & Ruch, T. C. (1947*a*). Morphology of the spinal cord, spinal nerves, caudal plexus, tail segmentation, and caudal musculature of the spider monkey. *Yale J. biol. Med.* **19**, 345–377.

Chang, H. T. & Ruch, T. C. (1947*b*). Topographical distribution in the thalamus of the spider monkey. *J. Anat., Lond.*, **81**, 150–164.

Clark, W. E. Le Gros (1932). A morphological study of the lateral geniculate body. *Brit. J. Ophthal.* **16**, 264–284.

Gardner, E. & Cuneo, H. M. (1945). Lateral spinothalamic tract and associated tracts in man. *Arch. Neurol. Psychiat., Chicago*, **53**, 423–430.

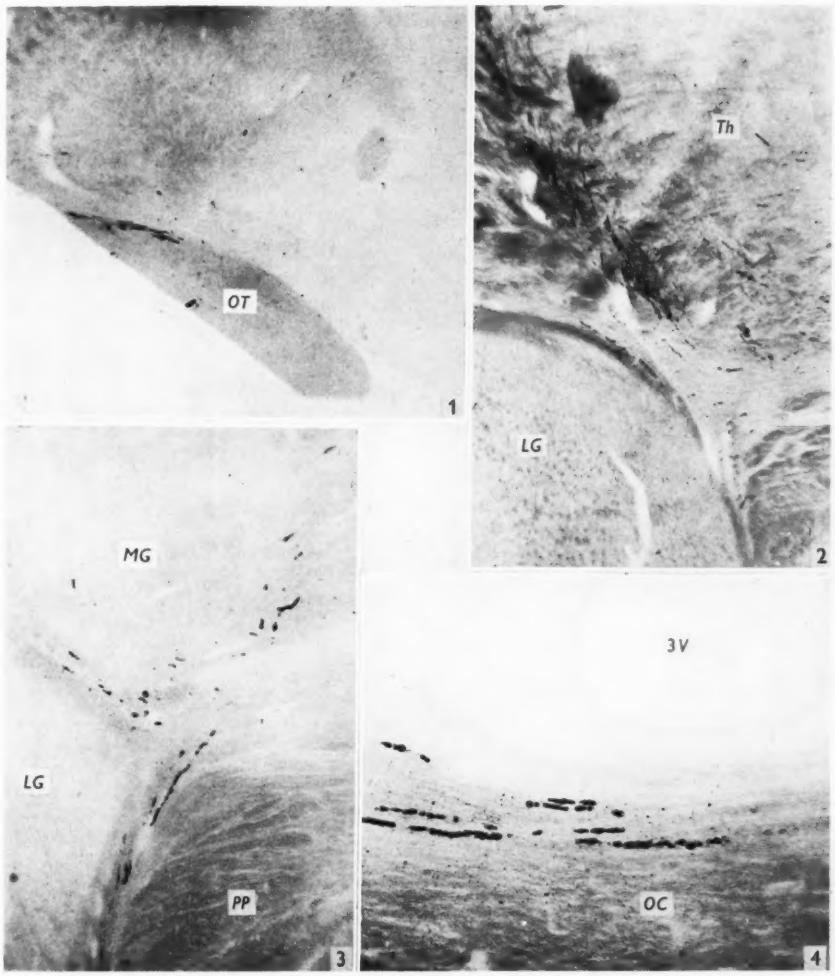
Gerebtzoff, M. A. (1939). Contribution anatomo-expérimentale à l'étude des commissures supra-optique. *J. belge Neurol. Psychiat.* **39**, 320–335.

Glees, P. (1944). The contribution of the medial fillet and strio-thalamic fibres to the dorsal supra-optic decussation of the lateral fillet. *J. Anat., Lond.*, **78**, 113–117.

Gudden, B. von (1870). Experimentale Untersuchungen über das peripherische und centrale Nervensystem. *Arch. Psychiat. Nervenkr.* **2**, 693–723.

Kuhlenbeck, H. & Miller, R. N. (1942). The pretectal region of the rabbit's brain. *J. comp. Neurol.* **76**, 323–365.

Magoun, H. W. & Ranson, M. (1942). The supraoptic decussations in the cat and monkey. *J. comp. Neurol.* **76**, 435–459.



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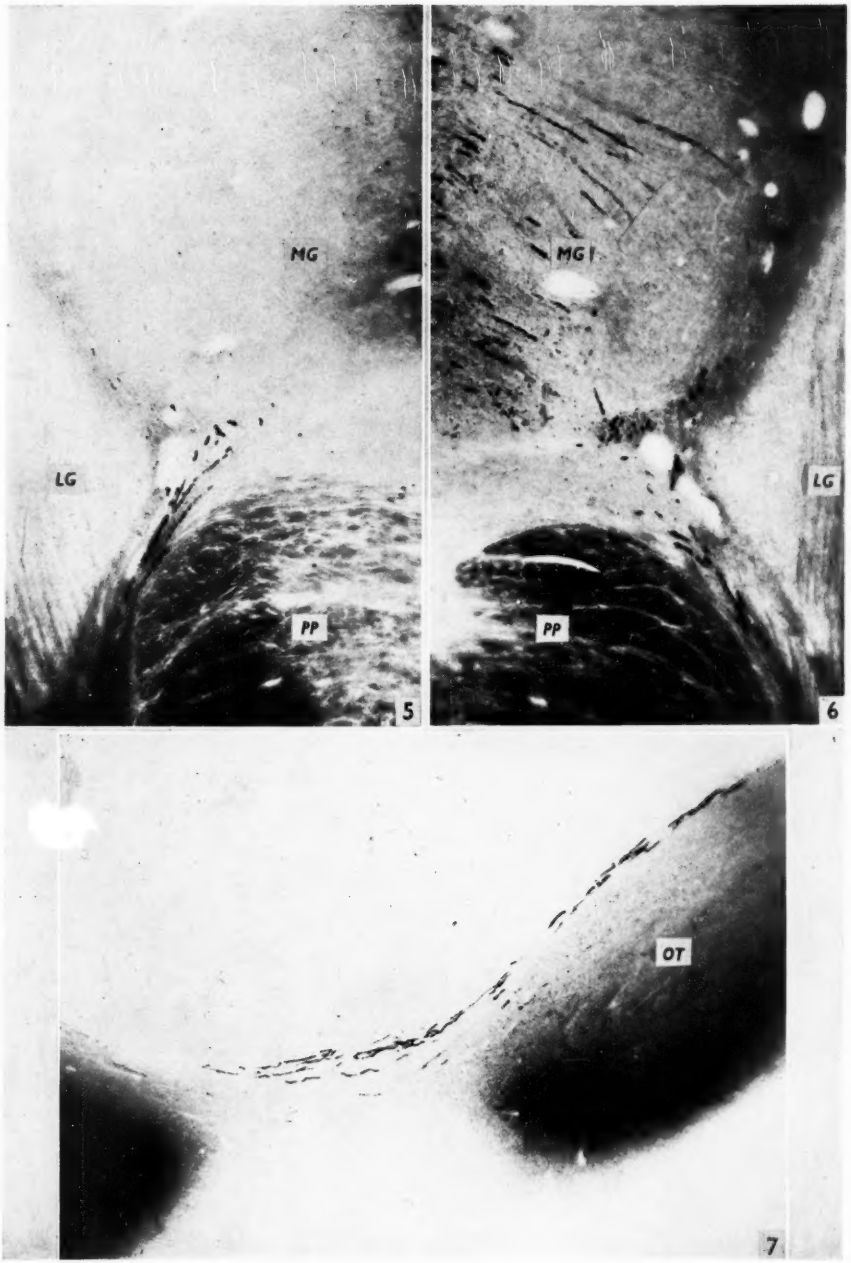

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METTLER, F. A. (1945). Pallidal isolation in the monkey: chronic physiological and anatomical results. *J. comp. Neurol.* **83**, 93–111.

MINKOWSKI, M. (1920). Über den Verlauf, die Endigung und die zentrale Repräsentation von gekreuzten und ungekreutzen Sehnervenfassern bei einigen Saugetieren und beim Menschen. *Schweiz. Arch. Neurol. Psychiat.* **6**, 201–252; **7**, 268–303.

OBERSTEINER, H. (1912). *Anleitung beim Studium des Baues der nervösen Zentralorgane im gesunden und kranken Zustande*, 5th ed., xix, 764 pp.

PAPEZ, J. W. (1938). Thalamic connections in a hemidecorticate dog. *J. comp. Neurol.* **69**, 103–120.

PAPEZ, J. W. & RUNDLES, R. W. (1938). Thalamus of a dog without a hemisphere due to a unilateral congenital hydrocephalus. *J. comp. Neurol.* **69**, 89–102.

RIOCH, D. McK. (1931). Studies on the diencephalon of carnivora. III. Certain myelinated fiber connections of the diencephalon of the dog (*Canis familiaris*), cat (*Felis domestica*), and aevisa (*Crossarchus obscurus*). *J. comp. Neurol.* **53**, 319–388.

SCHAFER, E. A. & SYMINGTON, J. (1908). Neurology. In *Quain's Elements of anatomy*. London: Longmans, Green and Co., 11th ed., vol. III, ix, 421 pp.

SOLNITZKY, O. & HARMAN, P. J. (1943). The lateral geniculate complex in the spider monkey, *Ateles ater*. *Yale J. biol. Med.* **15**, 615–639.

SWANK, R. L. & DAVENPORT, H. A. (1935). Chlorate-osmic-formalin method for staining degenerating myelin. *Stain Tech.* **10**, 84–90.

TILNEY, F. & RILEY, H. A. (1938). *The Form and Functions of the Central Nervous System*, 3rd ed. New York: Hoeber Co., xxxvi, 851 pp.

TSANG, Y.-C. (1940). Supra- and post-optic commissures in the brain of the rat. *J. comp. Neurol.* **72**, 535–567.

WEAVER, T. A. JR. (1937). Anatomic relations of the commissures of Meynert and Gudden in the cat. *J. comp. Neurol.* **66**, 333–347.

WOLLARD, H. H. & HARPMAN, A. (1939). The cortical projection of the medial geniculate body. *J. Neurol. Psychiat.* **2**, 33–44.

EXPLANATION OF PLATES

All photographs are unretouched

PLATE 1

Fig. 1. Degeneration in the dorsomedial fascicle of the optic tract; spinal transection at Ca_1 (SMS 13). 20×. *OT* = optic tract.

Fig. 2. Degenerated fibres in the neighbourhood of nucleus pregeniculatus (SMS 17, after a spinal transection at Ca_1). 20×. *LG* = lateral geniculate body, *Th* = thalamus.

Fig. 3. Degenerated fibres passing over the dorsolateral aspect of the pes pedunculi (SMS 14, spinal hemisection at Ca_1). 20×. *LG* = lateral geniculate body, *MG* = medial geniculate body, *PP* = pes pedunculi.

Fig. 4. Degenerated fibres in Gudden's commissure (SMS 14, spinal hemisection at Ca_1). 42×. *OC* = optic chiasma, 3*V* = 3rd ventricle.

PLATE 2

Fig. 5. Showing the absence of degenerated fibres traversing the medial geniculate body on the left side and the presence of degenerated fibres in the dorsolateral aspect of the pes pedunculi. SMS 18. 20×. *LG* = lateral geniculate body, *MG* = medial geniculate body, *PP* = pes pedunculi.

Fig. 6. Taken from the same section as Fig. 5, showing the pre-decussational stretch of the degenerated fibres passing through the medial geniculate body to the medial border of the optic tract on the operated right side. SMS 18. 20×.

Fig. 7. Section taken at the level of the posterior end of the optic chiasma showing the degenerated fibres in Gudden's commissure after unilateral anterolateral chordotomy at C_3. SMS 18. 28×.

ALKALINE PHOSPHATASE IN THE UTERINE EPITHELIUM OF THE RAT

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INTRODUCTION

Recently it has been shown by a number of workers that the phosphatase activity of certain parts of the reproductive tract is under the control of the gonadal hormones.

Thus, in the dog (Huggins, Masina, Eichelberger & Wharton, 1939) and rhesus monkey (Gutman & Gutman, 1939) androgens promote the secretion of acid phosphatase by the prostate. In the castrated mouse the administration of oestrogens leads to a marked increase in the alkaline phosphatase content of the surface and glandular epithelium and circular muscle of the uterus (Atkinson & Elftman, 1946, 1947) and of the vaginal epithelium (Jeener, 1947). In the castrated monkey oestrogens increase and progesterone decreases the alkaline phosphatase activity of the endometrium (Atkinson & Engle, 1947). The same workers showed that this enzyme was present in high concentration in the human endometrium during the proliferative (follicular) phase of the menstrual cycle, but was absent in the secretory (luteal) phase. Pritchard (1948) found that alkaline phosphatase disappeared from the uterine epithelium of the pregnant rat during the active life of the decidua, but reappeared in the latter third of pregnancy.

These observations, though they substantiate the thesis that the phosphatases of the reproductive tract are controlled by the gonadal hormones, give little information as to the precise cytochemical changes involved, or their significance.

It was therefore decided to investigate in detail the distribution and activity of alkaline phosphatase in the uterine epithelium of the rat: (*a*) at different stages of normal reproductive life, (*b*) following ovariectomy, and (*c*) following the administration of stilboestrol and progesterone.

It was hoped that by these means the precise effects of the ovarian hormones on the alkaline phosphatase activity of the uterine epithelium might be determined, and a basis provided for further research on the significance of this enzyme in reproduction.

MATERIAL AND METHODS

Ninety-two piebald and albino rats of the Lister strain were used in the investigation. Their uteri were examined for alkaline phosphatase activity at the following stages of normal reproductive life, and after the following experimental procedures:

(*a*) Before puberty (seven animals).
(*b*) During normal oestrous cycles (twenty-four animals).
(*c*) During normal pregnancy (twenty-eight animals).
(*d*) During lactation (three animals).

(*e*) During pseudopregnancy (one animal).
(*f*) During anoestrus (two animals).
(*g*) After bilateral ovariectomy (six animals).
(*h*) After the administration of stilboestrol (ten animals).
(*i*) After the administration of progesterone (eleven animals).

Further details will be found in the section of the paper dealing with results.

The timing of the phases of the oestrous cycle was determined from daily, and sometimes twice-daily, vaginal smears. The day after the discovery of spermatozoa in the smear was taken as the first day of pregnancy.

Ovariectomy was performed with aseptic precautions under ether anaesthesia through bilateral dorsal incisions. Both ovaries, uterine tubes, and small portions of the upper ends of the uterine horns, together with surrounding fat, were extirpated.

Stilboestrol was administered in oily solution by subcutaneous injection in single doses which varied from 50 to 400 γ in different animals. Progesterone was administered in similar fashion in single or repeated doses of 5 mg.

Histological technique

In the lactating animals one uterine horn was removed under ether anaesthesia; in the other cases the uteri were removed post-mortem.

The uteri were fixed for 24 hr. in three changes of absolute alcohol, embedded in paraffin, and cut at a standard thickness of 8 μ.

Sections were flattened and dried on the slide with a minimum of heating.

After removal of wax the slides were coated with a thin film of celloidin, passed to water, and then incubated for 2 hr. at 37° C., with frequent agitation, in the sodium glycerophosphate substrate.

The solution used differed slightly from that originally employed by Gomori (1939) and was made up as follows: 2 % sodium glycerophosphate (5 parts), 2 % calcium nitrate (5 parts), 1 % magnesium chloride (2 parts), 0·1 % sodium hydroxide (3 parts), distilled water (30 parts). This mixture had a pH of 9·5.

Following incubation the calcium phosphate precipitated at sites of alkaline phosphatase activity was converted into cobalt sulphide in the usual way. A dense black precipitate of cobalt sulphide was interpreted as indicating *intense* alkaline phosphatase activity at the site of deposition, a dark brown precipitate as *moderate* activity, and a light brown one as *slight* activity.

In the description of individual specimens, however, the words 'stained for phosphatase' will be used as meaning 'showed a precipitate of cobalt sulphide' or 'exhibited alkaline phosphatase activity'.

Terminology

In describing the localization of phosphatase activity in glandular and surface epithelial cells, the cytoplasm between the nucleus and the basement membrane will be termed the *basal* cytoplasm, and that between the nucleus and the free surface of the cell, the *distal* cytoplasm. The narrow zone of specialized eosinophilic cytoplasm immediately adjacent to the lumen will be termed the *distal band*.

RESULTS

This paper is primarily concerned with a description of the changes in alkaline phosphatase activity in the epithelial lining of the uterus, and with a correlation of these changes with the physiological activity of the uterus.

At one time or another most of the other tissues of the uterus also showed phosphatase activity in greater or lesser degree but, with a few exceptions, physiological correlations were difficult to establish. Only a brief general statement about phosphatase activity in these other tissues will therefore be given before passing to the detailed observations made on the surface epithelium.

Muscularis

Ten days after birth (rat 62*a*, Pl. 1, fig. 1) both circular and longitudinal coats stained for phosphatase. Staining was predominantly nuclear, and the nuclei of the circular coat were more heavily stained than those of the longitudinal coat. At the mesometrial and anti-mesometrial extremities of the uterus the nuclei of the circular coat were particularly heavily stained.

At 21 days (rat 53) staining in these regions was greatly reduced in intensity.

At 28 days (rat 62*b*, Pl. 1, fig. 2) the muscular coats were completely unstained, except at the mesometrial pole where a small patch of the circular coat showed moderately stained nuclei. All older animals were similar to this one in respect of phosphatase activity in the muscularis.

Stroma

On the basis of phosphatase activity two regions could be differentiated (Pl. 1, figs. 1, 2). The nuclei of the stromal cells in a zone beneath the surface epithelium and around the glands always showed some activity, which varied in intensity from slight to moderate at different phases of the life history of the animal. The nuclei of the outer stromal cells lying adjacent to the circular muscle coat were always inactive. The stromal cytoplasm was inactive.

There was some evidence that the activity of the nuclei of the inner part of the stroma varied inversely with the intensity of oestrogenic stimulation, but the changes were too slight for this to be claimed with any certainty.

Blood vessels. Capillary endothelium invariably showed intense nuclear and cytoplasmic phosphatase activity (Pl. 1, fig. 4; Pl. 2, fig. 6). The nuclei in the adventitial coat of the mesometrial arteries were also intensely active in all the specimens examined.

Leucocytes. During the normal oestrous cycle polymorphonuclear leucocytes were always present between the stromal cells, and in late oestrus and metoestrus they were present between the epithelial cells also. Their nuclei and cytoplasm always showed intense phosphatase activity.

Glandular epithelium. The nuclei always showed moderate to heavy activity (Pl. 1, fig. 3) in the nuclear membrane, nucleoli and chromatin granules. The basal cytoplasm was invariably inactive, but the distal cytoplasm usually showed diffuse activity, varying in intensity both from cell to cell in the same tubule, and from animal to animal.

In many cells the distal cytoplasmic band showed intense phosphatase activity. The secretion within the glandular lumen was also intensely active.

There was some evidence that phosphatase activity, in nuclei and cytoplasm alike, was increased during oestrogenic stimulation, but as in the case of the nuclei of the stromal cells, the results were not definite enough to establish this with certainty.

SURFACE EPITHELIUM

The uterine cavity of the rat is lined by a single layer of non-ciliated columnar epithelial cells supported, at most times, by a well-marked basement membrane.

The height of the epithelium undergoes marked changes during the oestrous cycle and pregnancy (Allen, 1931), depending chiefly on the level of oestrogenic stimulation (Atkinson & Hooker, 1945). During pro-oestrus the height of the epithelium is inversely related to the degree of uterine distention.

In late oestrus the epithelium undergoes degenerative changes associated with the appearance of large vacuoles in the cytoplasm, and polymorphonuclear leucocytes between the cells (Long & Evans, 1922). The basement membrane is lost at this stage.

During metoestrus the epithelium is repaired and mitotic figures make their appearance. These are seen again in the later stages of dioestrus.

Secretory activity is high during pro-oestrus when the secretion distends the uterus to about three times its ordinary size.

These morphological changes in the epithelium, while insufficient for certain diagnosis of a particular phase of the oestrous cycle, provide a useful check on the deductions made from the vaginal smear. For this reason an estimate of the height of the epithelium (obtained by averaging six measurements made with a micrometer eyepiece on portions of the epithelium selected at random) will precede the description of phosphatase activity in each specimen, and the presence of vacuolation, leucocytic infiltration or secretory activity will be noted wherever these phenomena were observed.

Generally, the nuclei and distal cytoplasm of the surface epithelium stained for phosphatase. The basal cytoplasm was always unstained. The distal cytoplasm, apart from the distal band, stained diffusely and rather unevenly in most specimens. The distal band, when stained, appeared homogeneous, but close inspection showed that in reality it was finely granular.

Before puberty

Ten days after birth (rat 62*a*, Pl. 1, fig. 1; Pl. 2, fig. 7) the uterine epithelium averaged 11 μ in height. Neither nuclei nor cytoplasm stained for phosphatase after the standard incubation time (2 hr.).

At 3 weeks (rat 53) and 4 weeks (rat 62*b*, Pl. 1, fig. 2; Pl. 2, fig. 8) the height of the epithelium was unchanged, but now the nuclei were faintly stained for phosphatase, although the cytoplasm remained unstained.

At 6 weeks, in one specimen (rat 54, Pl. 2, fig. 9), the epithelium was 16 μ in height, in another (rat 71) it was still only 11 μ. In both specimens the nuclei stained faintly. Near the free border of the cells the cytoplasm showed a thin but distinct

line of heavy staining (distal band). The remainder of the distal cytoplasm showed only faint, diffuse staining. The basal cytoplasm in these, as in all other specimens examined, was unstained.

At 2 months (rat 62*c*) the epithelium was considerably taller (23μ). The distribution and intensity of phosphatase staining, however, were unaltered.

At 3 months, almost all animals investigated had reached puberty, and oestrous cycles had begun. Rat 55, however, was still in anoestrus. Its epithelium was 20μ in height. The nuclei stained with moderate intensity. The distal band was a little wider, and stained more intensely than in previous specimens. The remainder of the distal cytoplasm stained with moderate intensity in a diffuse manner.

It appears from these observations that the surface epithelium of the uterus begins to increase markedly in height about 6 weeks after birth, and that intense phosphatase activity appears at the same time in a thin cytoplasmic band just beneath the free border of the cells. From the age of 3 weeks some activity is also present in the nuclei and distal cytoplasm generally.

Oestrous cycle

Anoestrus. Rat 44, aged 6 months, showed no signs of cyclical variations in the cellular content of the vaginal smear, and was judged to be either in anoestrus or pseudopregnant. Examination of the uterus showed that leucocytes were absent from the stroma, and that the stromal nuclei were small and ovoid and quite unlike the large, spherical, vesicular elements seen in pseudopregnancy. This rat was therefore classified as in anoestrus.

The uterine epithelium was 15μ in height. Its nuclei stained for phosphatase with moderate intensity and the distal cytoplasm showed only faint diffuse staining. The distal band was stained in only a small proportion of the cells.

In rat 78, judged to be in anoestrus on similar evidence, the epithelium stained for phosphatase in a similar manner.

Pro-oestrus. Rat 76 gave a vaginal smear consisting solely of nucleated cells. The uterus was hyperaemic but not markedly distended. It was judged to be in early pro-oestrus.

The epithelium was very tall (30μ), due to a marked increase in the amount of the distal cytoplasm. The nuclei were regularly arranged in the basal part of the cell.

The nuclei stained very faintly, but the cytoplasm generally was unstained. The distal band, however, was wider than at any other stage of the oestrous cycle (Pl. 2, fig. 12) and stained intensely. A small amount of heavily stained secretion was present within the uterine lumen.

Rat 117 was also in pro-oestrus. Its uterus was moderately distended, but the epithelium was still tall (25μ).

The nuclei stained a little more heavily than in the previous specimens, and the distal cytoplasm was faintly stained. The distal band was very wide, and in places heavily stained secretion could be seen passing into the dilated uterine lumen (Pl. 2, fig. 11), where considerable quantities of secretion had already accumulated (Pl. 2, fig. 5).

The uterus in rat 118 was greatly distended. The epithelium was now only 8μ in height and the nuclei filled the greater part of the cell. These stained moderately

heavily, as did the distal cytoplasm. The distal band was wide, and very heavily stained, as before, and great quantities of secretion could be seen passing into the lumen (Pl. 2, fig. 10). Rat 119 showed similar features.

Oestrus. Rat 99 gave a vaginal smear in which cornified elements were mixed with the nucleated cells. It was judged to be in early oestrus. The uterus was greatly distended and its epithelium was very low (8 μ). Its phosphatase activity was very similar to that of the previous specimen.

In rat 47, which gave a similar type of vaginal smear, uterine distention had passed off, and the epithelium was considerably taller (15 μ). Phosphatase activity was much the same as in the previous specimen.

Rat 62*d* (Pl. 2, fig. 6; Pl. 3, fig. 13) gave a vaginal smear consisting solely of cornified cells. The uterus was undistended and the epithelium was very tall (27 μ). Many of the cells showed large vacuoles, as big as, or bigger than, the nuclei, and containing granular debris. In other morphological respects the epithelium was not markedly different from that of early pro-oestrus.

The nuclei and distal cytoplasm stained moderately heavily. The distal band was heavily stained, but was not quite so wide as in previous specimens, and there was much less secretory activity. Within the vacuoles the granular debris was moderately heavily stained.

Rat 73 showed similar features to that last described. Its vaginal smear contained numerous active spermatozoa.

Rat 74 (Pl. 3, fig. 14) was in late oestrus. Its epithelium was 30 μ in height and, in addition to vacuolation, showed heavy infiltration with polymorphonuclear leucocytes.

The nuclei stained either moderately or heavily for phosphatase. The distal cytoplasm was moderately heavily stained. The distal band was conspicuous, but was a little thinner than before, and secretory activity was slight.

Rats 1 and 77 were similar.

Metoestrus. Rat 79 gave a vaginal smear containing a mixture of cornified cells and leucocytes. The epithelium was 19 μ in height, and vacuolar degeneration and leucocytic infiltration were much less in evidence than during late oestrus.

The nuclei and distal cytoplasm stained moderately heavily. Near the nucleus the latter showed an intensely stained reticulum in many of the cells (Pl. 3, fig. 15) identical in situation and shape with the Golgi element as visualized after silver impregnation. This body was not seen, after phosphatase staining, at any other stage of the oestrous cycle. The distal band was further diminished in thickness as compared with previous specimens.

Similar observations were made on rats 45, 56, 80 and 82, except that in these staining of the Golgi element was capricious.

Dioestrus. Rat 84 was judged from the character of the vaginal smear to be in early dioestrus. The epithelium was essentially similar to that of metoestrus except that the Golgi element was no longer visible after phosphatase staining. Rat 86 was similar.

In rat 46, later in dioestrus, the epithelial height had fallen to 15 μ. The nuclei were moderately heavily stained as before, but the distal cytoplasm stained only faintly. The distal band was thinner than at any other stage of the oestrous cycle (Pl. 3, fig. 16). Rat 62*c* gave a similar appearance.

It was concluded from this series of observations: (1) that during pro-oestrus and early oestrus the distal band shows great phosphatase activity which gradually diminishes during the later stages of the cycle, and almost disappears during anoestrus; (2) that during the stage of uterine distention (late pro-oestrus and early oestrus) great quantities of phosphatase are secreted into the uterine lumen; and (3) that phosphatase activity in the nucleus and distal cytoplasm (excluding the distal band) varies inversely with that of the distal band, being lowest during pro-oestrus and highest during dioestrus.

PREGNANCY

The uterine epithelium during pregnancy was studied in two series of rats. In the first series one rat was killed on each day of pregnancy up to full term. In the second series fewer rats were employed and these were killed at intervals of 3 or 4 days.

First 4 days. From the time of copulation up to and including the 4th day the changes in the epithelium, both as regards morphological characters and phosphatase activity, were similar to those of late oestrus, metoestrus and dioestrus already described.

5th–12th days. On the 5th day (rat 58) the epithelial height had fallen to 10 μ, except at the mesometrial end of the lumen where taller cells were found. In this latter situation the nucleus and distal cytoplasm stained faintly for phosphatase, and a thin, moderately heavily stained distal band was present. In the rest of the epithelium the cells did not stain at all. Rat 5 was similar.

On the 6th day (rat 43) the epithelium had a similar height. The nuclei everywhere stained faintly, but the cytoplasm, including the distal band, was unstained.

On the 8th day (rat 87) the epithelium was a little taller (15 μ), but neither nuclei nor cytoplasm was stained (Pl. 3, fig. 17).

From the 9th until the 12th day the chief changes noted were an increasing irregularity of the outline of the lumen and a 'hillocky' appearance of the epithelium. The cells remained unstained.

13th day to full term (21st day). On the 13th day (rat 97) the epithelium was 15 μ in height. Both nuclei and cytoplasm were faintly stained for phosphatase. A thin intensely stained distal band was present in about one-half of the cells. In rat 13, at the same day of pregnancy, the epithelium was similar except that the distal band was unstained.

On the 14th day (rat 14) the distal band stained intensely in all cells.

On the 15th day (rat 15, Pl. 3, fig. 18) the epithelium was taller (20 μ). The nuclei were, for the most part, at the basal end of the cells as in pro-oestrus. Both nuclei and distal cytoplasm were moderately heavily stained. The distal band, however, was very wide and stained intensely. Moreover, great quantities of the enzyme were seen in process of secretion into the uterine lumen, again suggestive of pro-oestrus or early oestrus.

There were similar appearances in the epithelium throughout the remainder of pregnancy.

Thus during pregnancy the uterine epithelium shows: (1) normal oestrous and dioestrous appearances during the first 4 days; (2) virtual disappearance of the enzyme between the 5th and 12th days; and (3) reappearance of the enzyme on the

13th day, with great activity in the distal band, and great secretory activity, lasting until full term.

LACTATION

Three animals only were investigated during lactation. On the 4th day (rat 113) the epithelium was similar to that seen in late dioestrus and on the 4th day of pregnancy.

On the 8th day (rat 115, Pl. 4, fig. 20) the epithelium was tall (20μ) and the nuclei were regularly arranged in the *distal* part of the cell. The nuclei stained moderately heavily as did the thin strip of distal cytoplasm between the nucleus and the distal band. The latter was fairly wide, and heavily stained, though not so intensely as in pro-oestrus.

On the 15th day (rat 116) the epithelium was not quite so tall (16μ), but the position of the nuclei, and the phosphatase staining, were comparable with those of the previous specimen.

PSEUDOPREGNANCY

This condition was not deliberately investigated, but in one animal (rat 81), which did not show cyclical variations in the vaginal smear, the nuclei of the stromal cells were large, spherical, and vesicular, as in early pregnancy and pseudopregnancy. The animal was not pregnant, and it was assumed that it was pseudopregnant, probably as the result of accidental stimulation of the cervix during the routine vaginal examination.

The uterine epithelium was moderately tall (15μ). The nuclei stained very faintly, and the cytoplasm, including the distal band, was quite unstained (Pl. 4, fig. 19). The epithelium was therefore similar to that of animals between the 6th and 12th days of pregnancy.

OVARIECTOMY

The uterine epithelium was examined in bilaterally ovariectomized animals at intervals from the 8th day until 4 months after operation.

On the 8th day (rat 59, Pl. 4, fig. 21) the epithelium was 10μ in height. The nuclei were moderately heavily stained. The distal cytoplasm stained faintly apart from the distal band, which was thin, but heavily stained, in some cells. Other cells showed either faint staining or complete absence of staining in the distal band. Similar appearances were seen on the 9th day (rat 100).

At the 15th day (rat 60) the epithelium was 9μ in height. While the nuclei and general cytoplasm stained as before, the distal band was very thin and stained with only moderate intensity. In some cells it was absent altogether.

Rat 68, at the 14th day, was similar.

Little change was seen either in the morphological characters or in the phosphatase staining of the epithelium, at longer intervals after operation. After 4 months (rat 63, Pl. 4, fig. 22) the nuclei stained with faint to moderate intensity, and the distal band, though extremely thin, still stained heavily.

Thus, following ovariectomy, there is a marked reduction in phosphatase activity in the uterine epithelium, although some activity persists for at least 4 months. Fifteen days after ovariectomy the epithelium is very similar to that seen in anoestrus and in animals about 6 weeks of age.

STILBOESTROL

Immature animals. In rat 71, 6 weeks after birth the uterine epithelium was 11 μ high, the nuclei and distal cytoplasm stained faintly, and a thin moderately heavily stained distal band was present.

Rat 71 *a*, a litter mate, was given 50 γ stilboestrol in oil by subcutaneous injection. After 24 hr. its uterine epithelium was 26 μ in height. The nuclei and distal cytoplasm stained moderately heavily. The distal band was very much wider than in the control, and stained intensely, and some heavily stained secretion was present within the lumen.

Rat 72, another litter mate, was given a similar dose of stilboestrol. Forty-eight hours later the uterus showed moderate pro-oestrous distention and its epithelium was very tall (30 μ). The nuclei and distal cytoplasm were less heavily stained than in the previous specimen, but the distal band was even wider, and great quantities of secretion were seen passing into the uterine lumen (Pl. 4, fig. 23).

Rat 72 *a*, yet another litter mate, was given a similar dose of stilboestrol. Four days later the uterus was undistended and its epithelium was 24 μ in height, and showed commencing vacuolar degeneration. The nuclei and distal cytoplasm stained moderately heavily, and the distal band was wide and intensely stained, but there was little secretory activity.

Ovariectomized animals. In rat 69, 6 weeks after bilateral ovariectomy, the epithelium was 7 μ in height. The nuclei stained faintly, the distal cytoplasm was unstained, and the distal band, when present, was very thin and only moderately heavily stained. Rat 70, ovariectomized for a similar length of time, was given 50 γ stilboestrol. Forty-eight hours later the uterus was moderately distended and the epithelium was 25 μ in height (Pl. 4, fig. 24). The nuclei stained with faint to moderate intensity, and the distal cytoplasm was faintly stained. The distal band was wide and very heavily stained and there was great secretory activity.

Rat 70 *a* was treated similarly, except that it was examined 4 days after the injection of stilboestrol. The uterus was undistended and vacuolar degeneration and leucocytic infiltration of the epithelium were marked. The cells were 25 μ high. The nuclei and distal cytoplasm were moderately heavily stained. The distal band was wide, and intensely stained, but secretory activity was minimal.

Rat 51, ovariectomized 3 months previously, was given 200 γ stilboestrol. Four days later the uterus was undistended. The epithelium was extremely tall (40 μ) and there was no sign of vacuolar degeneration or leucocytic infiltration. The nuclei stained very faintly and the cytoplasm was unstained. In some cells the distal band was wide and intensely stained, but in others it was unstained. Very great quantities of heavily stained secretion, however, were present in the lumen. Rat 64, also ovariectomized 2 months previously, was given 400 γ stilboestrol. Four days later the uterine epithelium was in a similar condition to that just described.

Rat 67, ovariectomized 24 days previously, was given 200 γ stilboestrol. Three days later the uterus was extremely distended and the epithelium was only 7 μ in height. The nuclei stained faintly, but the cytoplasm, including the distal band, was completely unstained. Great quantities of secretion, however, were present within the lumen.

Thus 50 γ stilboestrol injected into either immature or ovariectomized rats, reproduces pro-oestrous and oestrous conditions in the uterine epithelium, both as regards morphological and phosphatase characters. With larger doses the enzyme disappears completely from many of the cells, presumably because it has all been secreted into the lumen.

PROGESTERONE

Rat 111 was found to be in pro-oestrus at the time of the routine vaginal smear. 5 mg. of progesterone in oil were injected subcutaneously and the animal was killed 2 days later. The uterine epithelium was 16 μ in height. The nuclei and distal cytoplasm stained moderately heavily, and the distal band was moderately wide and intensely stained. The cells, in fact, stained as in early dioestrus.

Rat 101, also in pro-oestrus, was given 5 mg. progesterone and a similar dose 2 days later when killed. Five days after the first injection the epithelium was 14 μ in height. The nuclei stained extremely faintly, and the cytoplasm, including the distal band, was completely unstained (Pl. 4, fig. 25).

Rat 112 was also in pro-oestrus. It was treated in a similar manner to the last named, except that it was not killed until 8 days after the first injection. The epithelium was 15 μ high, and no phosphtase was detectable in either nucleus or cytoplasm.

Rats 120, 121 and 122 were treated similarly at pro-oestrus. They were killed 11 days after the first injection. Rat 120 showed similar appearances to those just described. Rats 121 and 122, however, were in pro-oestrus and showed the characteristic wide heavily stained distal band and minimally stained nucleus and general cytoplasm.

Evidently progesterone causes the complete disappearance of phosphatase from the nucleus and cytoplasm, the effect beginning to wear off about 9 days after the last injection.

Rats 50 and 66 were injected with 5 mg. progesterone 3 months after ovariectomy, and killed 5 days later. The epithelium showed no significant differences from that of untreated ovariectomized animals, so it would appear that progesterone leads to a disappearance of epithelial phosphatase only when it acts on cells recently under the influence of oestrogens.

For convenience of cross-reference the more important observations on the morphological characters and phosphatase activity of the uterine epithelium at the various stages detailed above have been summarized and tabulated (Table 1).

Table 1

Stage or experiment	Epithelial height (μ)	Nuclear phosphatase activity	Distal cytoplasmic phosphatase activity	Distal band phosphatase activity	Secretory activity	Remarks
Before puberty:						
10 days	11	0	0	0	0	—
21 days	11	+	0	0	0	—
28 days	11	+	0	0	0	—
6 weeks	11–16	+	±	+	0	—
8 weeks	23	+	±	+	0	—
13 weeks	20	++	+	++	0	Most animals of this age undergoing oestrous cycles

Table 1 (*continued*)

Stage or experiment	Epithelial height (μ)	Nuclear phosphatase activity	Distal cytoplasmic phosphatase activity	Distal band phosphatase activity	Secretory activity	Remarks
Anoestrus	15	+ +	+	±	0	—
Pro-oestrus:						
Early	30	±	0	+ + + +	+	Regularly arranged basal nuclei
Middle	25	+	±	+ + + +	+ + +	—
Late	8	+ +	+	+ + + +	+ + + +	Grossly distended uterus
Oestrus:						
Early	8	+ +	+	+ + + +	+ + + +	Grossly distended uterus
Middle	15	+ +	+	+ + + +	+ + + +	—
Late	27	+ +	+ +	+ + +	+ +	Vacuolar degeneration
Very late	30	+ + to + + +	+ +	+ +	+	Vacuolar degeneration and polymorphic infiltration
Metoestrus	19	+ +	+ +	+ +	0	Golgi element heavily stained
Dioestrus:						
Early	19	+ +	+ +	+ +	0	—
Late	15	+ +	+	+	0	—
Pregnancy:						
0–4 days	—	—	—	—	—	Similar to late oestrus, metoestrus and dioestrus
5–12 days	10–15	±	0	0	0	—
13–21 days	15–20	+ to + +	+ to + +	+ + + +	+ + + +	—
Lactation:						
4th day	—	—	—	—	—	Similar to dioestrus
8th day	20	+ +	+ +	+ +	0	Nuclei distal
15th day	16	+ +	+ +	+ +	0	Nuclei distal
Pseudopregnancy	15	±	0	0	0	—
Ovariectomy:						
8 days	10	+ +	±	0 to +	0	—
15 days	9	+ +	±	0 to +	0	—
4 months	7	+ to + +	±	+	0	—
Stilboestrol to immature rats:						
50γ. 24 hr.	26	+ +	+ +	+ + +	+ +	—
50γ. 48 hr.	30	+	+	+ + + +	+ + + +	—
50γ. 96 hr.	24	+ +	+ +	+ + + +	+	Vacuolar degeneration
Stilboestrol to ovariectomized rats:						
50γ. 48 hr.	25	+ to + +	+	+ + + +	+ + + +	—
50γ. 96 hr.	25	+ +	+ +	+ + + +	±	Vacuolar degeneration and leucocytic infiltration
200–400γ. 96 hr.	40	±	0	0 to + +	+ + + +	No degeneration
Progesterone at pro-oestrus:						
5 mg. 48 hr.	16	+ +	+ +	+ +	0	—
10 mg. 5 days	14	±	0	0	0	—
10 mg. 8 days	15	0	0	0	0	—
10 mg. 11 days (*a*)	15	0	0	0	0	—
10 mg. 11 days (*b*)	30	±	±	+ + + +	+ +	Effect worn off. Animal pro-oestrous again
Progesterone to ovariectomized animals	—	—	—	—	—	Same as untreated controls

0, no phosphatase activity; ±, minimal activity; +, slight activity; + +, moderate activity; + + +, heavy activity; + + + +, very heavy activity.

DISCUSSION

This investigation has shown that the distribution and intensity of alkaline phosphatase activity within the surface epithelium of the uterus of the rat exhibits gross variations during normal reproductive life, following castration, and after the administration of stilboestrol and progesterone. Variations in the activity of the enzyme within the other tissues of the uterus are of a minor character, and of doubtful significance.

In the surface epithelium the alkaline phosphatase activity of the distal band of the cytoplasm shows the greatest variation. The changes in the nucleus and distal cytoplasm, apart from the distal band, are definite but much less pronounced. The basal cytoplasm never shows phosphatase activity. Maximal activity in the distal band is associated with secretion of the enzyme into the uterine lumen.

The changes in cytoplasmic activity are largely independent of those occurring in the nucleus. This is not surprising for, as was discussed in a previous paper (Pritchard, 1948), the cytoplasmic phosphatases appear to be chiefly concerned with functional changes in mature cells such as are involved, for example, in secretory activity, while the nuclear phosphatases are more concerned in the metabolic exchanges associated with cell division and differentiation.

The results summarized in Table 1 show that the effects of hormonal administration and ovariectomy on the distribution and activity of the enzyme in the uterine epithelium are closely paralleled by the changes occurring at certain stages of normal reproductive life.

Thus stilboestrol produces changes in the phosphatase activity of the uterine epithelium virtually identical with those found at pro-oestrus and in late pregnancy, when there is every reason for supposing that the endogenous production of oestrogens reaches a high level (Selye & McKeown, 1935). Progesterone, on the other hand, reproduces the conditions found in early pregnancy and pseudopregnancy when the endogenous production of progesterone is known to be at a high level (Atkinson & Hooker, 1945). There can be little doubt, therefore, that the naturally occurring variations in phosphatase activity during the oestrous cycle and in pregnancy are determined by the levels of circulating oestrogens and progesterone.

Furthermore, the phosphatase activity of the uterine epithelium in the sexually immature animal is consistent with a rising but sub-threshold oestrogen level, and that of the anoestrous and castrated animal with a very low oestrogen level, which is in accordance with what might have been expected in view of the conclusion reached above.

The phosphatase activity of the epithelium during lactation, however, appears at first sight to be at variance with what might have been expected. Judging from the level of phosphatase activity alone, a moderate level of oestrogenic activity might be inferred.

The ease with which deciduomata may be produced during lactation in the rat (Corner & Warren, 1919; Long & Evans, 1922; Selye & McKeown, 1935; Lyon & Allen, 1938) suggests that the level of circulating oestrogen is very low. In the mouse also there is evidence that the oestrogen level is very low during lactation (Atkinson & Leathem, 1946).

It is probable, however, that the production of oestrogens during lactation varies from animal to animal. For instance, Crew & Mirskaia (1930) state that while oestrous cycles are normally in abeyance during lactation, they may be resumed if the number of young suckled is small. Again, Krehbiel (1941) found that, depending on the size of the litter, a pregnancy of normal duration may accompany lactation in some cases, in others implantation may be delayed and pregnancy prolonged, in others again pregnancy may fail with absorption of embryos. In this connexion it may be significant that the rats employed in this investigation all had small litters, which would be compatible with a moderate level of circulating oestrogen as suggested by the level of phosphatase activity.

The functional significance of these changes in uterine phosphatase activity has not yet been determined. The fundamental role of the phosphatases is no doubt that of assisting in the hydrolysis of organic phosphate esters (Moog, 1946), but as this type of chemical change occurs during the intermediary metabolism of carbohydrates, fats and proteins alike, and probably also in the transport of a wide variety of organic substances, as well as in the early stages of calcification, it is evident that the exact significance of variations in phosphatase activity cannot be inferred from purely histological studies. One can only hope that the results obtained by histochemical studies such as this will point the way to significant biochemical investigations. There is, for example, possibly a physiological significance in the fact that oestrogens cause not only a mobilization of phosphatase in the uterine epithelium of the rat but also of glycogen (Boettiger, 1946), while lipoids are sharply diminished (Bourg, 1930; Alden, 1946). Again, the secretion of great quantities of the enzyme into the distended lumen at oestrus may be associated in some way with the metabolism or motility of spermatozoa.

Whatever the underlying explanation may turn out to be, the fact that phosphatase activity in the uterus is so closely linked with ovarian function, makes it probable that the role of the enzyme in reproductive processes is an important one.

SUMMARY

1. The alkaline phosphatase activity of the uterine epithelium of the rat has been investigated histochemically in the immature animal, throughout the oestrous cycle, in pregnancy, pseudopregnancy, lactation, after castration and following the administration of stilboestrol and progesterone.

2. Gross variations in the activity of the enzyme were found, particularly in the distal band of cytoplasm bordering the uterine lumen.

3. The results indicate that these variations are determined by the level of circulating oestrogens and progesterone.

4. Oestrogens cause a great concentration of the enzyme in the distal band of the cytoplasm, and its secretion into the uterine lumen.

5. Progesterone causes the disappearance of the enzyme from all parts of the cell.

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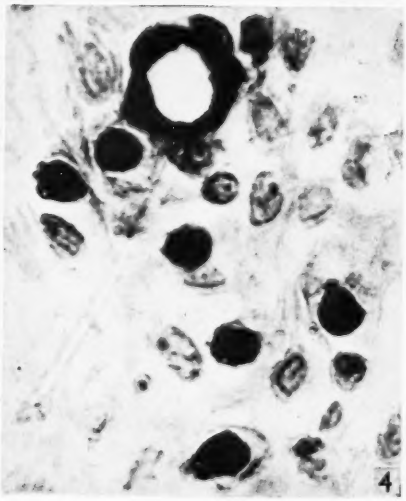
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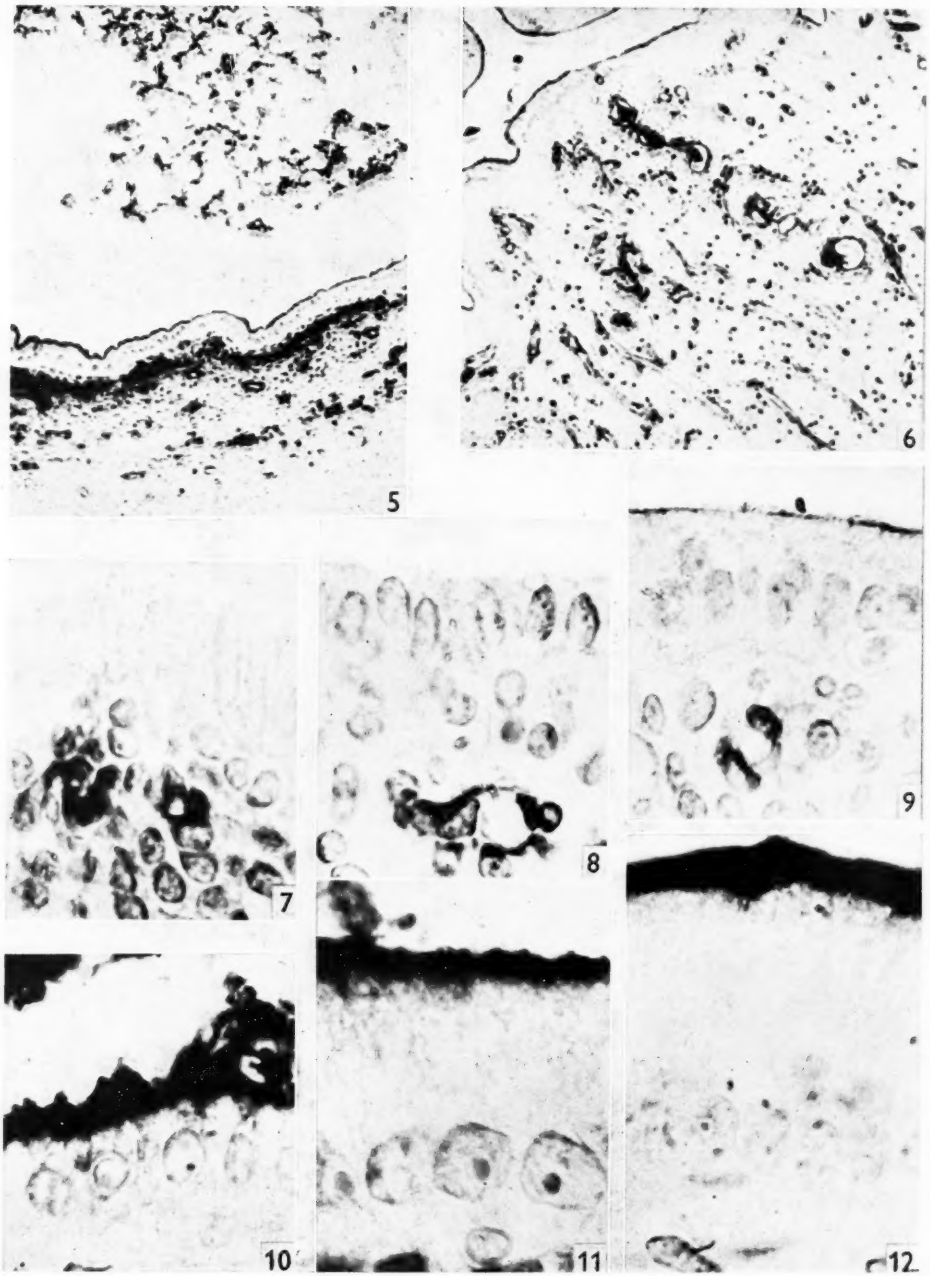
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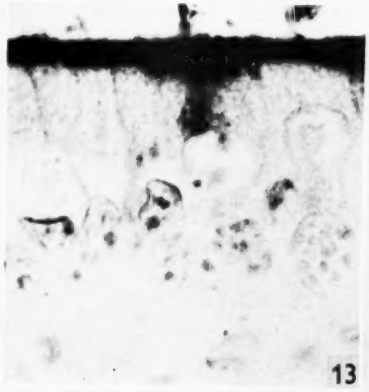
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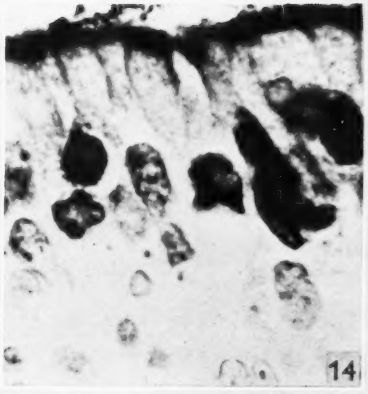
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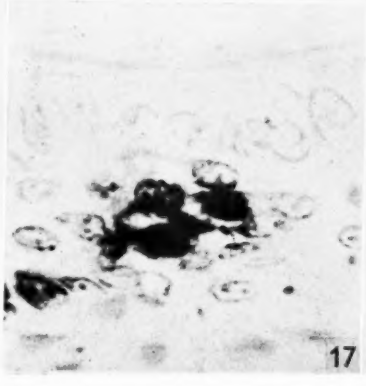
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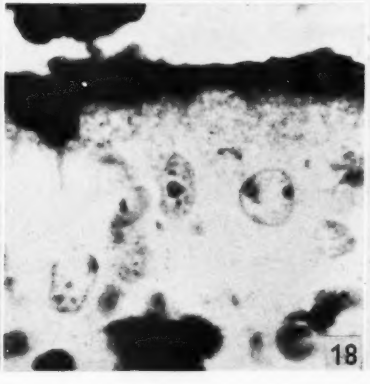
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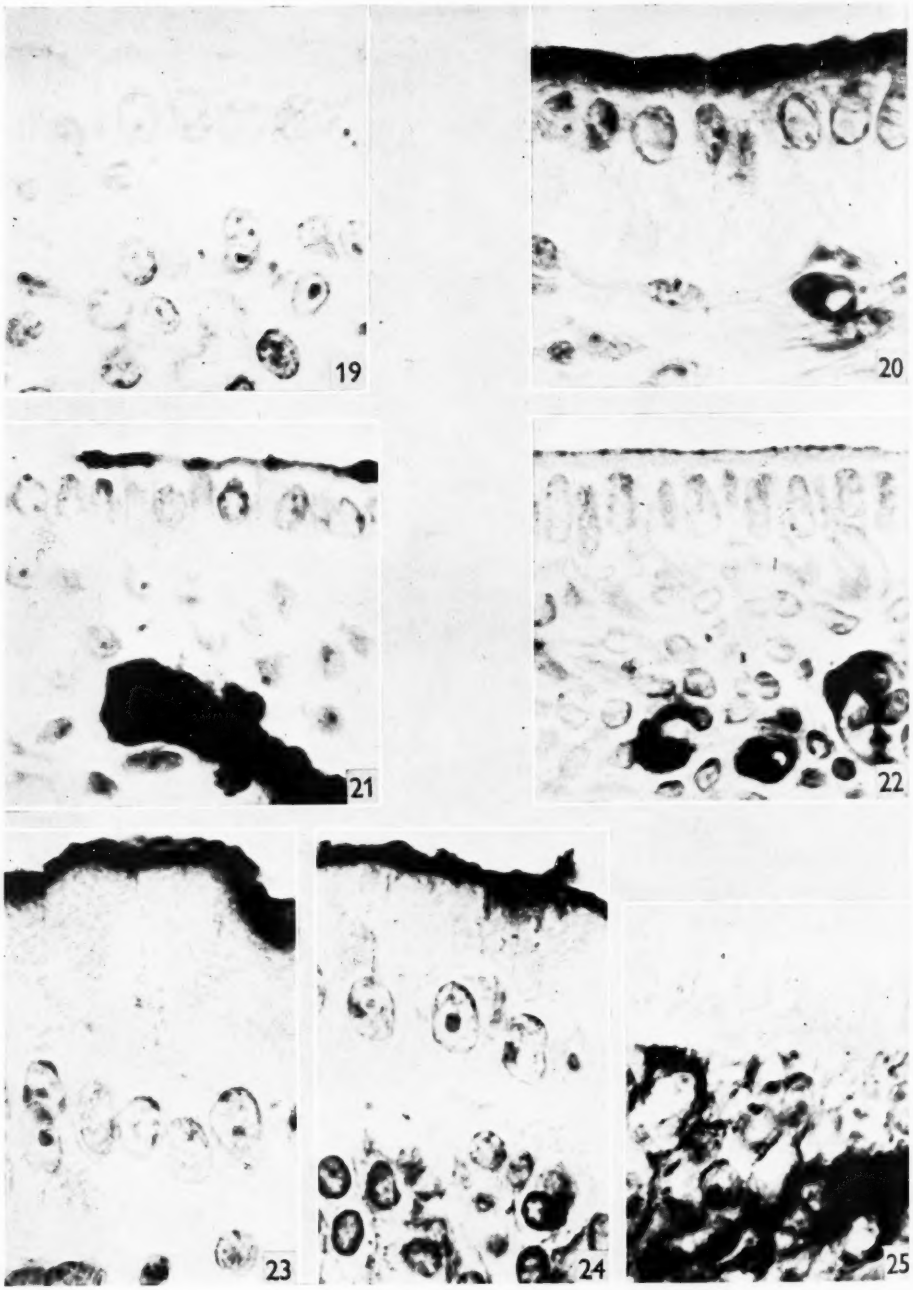

PRITCHARD—ALKALINE PHOSPHATASE IN THE UTERINE EPITHELIUM OF THE RAT

REFERENCES

ALDEN, R. H. (1946). Further studies on the alteration of epithelial fat in the rat uterus during the oestrous cycle, early pregnancy and in the treated and untreated castrate. *Anat. Rec.* **94**, 445–446.

ALLEN, W. M. (1931). Cyclical alterations of the endometrium of the rat during the normal cycle, pseudopregnancy and pregnancy. Production of deciduomata during pregnancy. *Anat. Rec.* **48**, 65–104.

ATKINSON, W. B. & ELFTMAN, H. (1946). Effect of steroid sex hormones on distribution of alkaline phosphatase in uterus of mouse. *Proc. Soc. exp. Biol., N.Y.*, **62**, 148–150.

ATKINSON, W. B. & ELFTMAN, H. (1947). Mobilization of alkaline phosphatase in the uterus of the mouse by oestrogen. *Endocrinology*, **40**, 30–36.

ATKINSON, W. B. & ENGLE, E. T. (1947). Studies in endometrial alkaline phosphatase during the human menstrual cycle and in the hormone-treated monkey. *Endocrinology*, **40**, 327–333.

ATKINSON, W. B. & HOOKER, C. W. (1945). The day to day level of oestrogen and progestin throughout pregnancy and pseudopregnancy in the mouse. *Anat. Rec.* **93**, 75–95.

ATKINSON, W. B. & LEATHEM, J. H. (1946). The day to day level of estrogen and progestin during lactation in the mouse. *Anat. Rec.* **95**, 147–158.

BOETTIGER, E. G. (1946). Changes in glycogen and water content of rat uterus. *J. cell. comp. Physiol.* **27**, 9–14.

BOURG, R. (1930). Les lipides de l'epithélium utérin et la triade oestrale dans la réaction de Zondek chez la souris et le rat impubères. *C.R. Soc. Biol., Paris*, **103**, 918–919.

CORNER, G. W. & WARREN, S. L. (1919). Influence of the ovaries upon the production of artificial deciduomata; confirmatory studies. *Anat. Rec.* **16**, 168–169.

CREW, F. A. E. & MIRSKAIA, L. (1930). The lactation interval in the mouse. *Quart. J. exp. Physiol.* **20**, 105–110.

GOMORI, G. (1939). Micro-technical demonstration of phosphatase in tissue sections. *Proc. Soc. exp. Biol., N.Y.*, **42**, 23–26.

GUTMAN, A. B. & GUTMAN, E. B. (1939). Acid phosphatase levels in prepubertal rhesus prostate tissue after testosterone propionate. *Proc. Soc. exp. Biol., N.Y.*, **41**, 277–281.

HUGGINS, C., MASINA, M. H., EICHELBERGER, L. & WHARTON, J. D. (1939). Quantitative studies of prostatic secretion; characteristics of normal secretion; influence of thyroid, suprarenal and testis extirpation and androgen substitution on prostatic output. *J. exp. Med.* **70**, 543–556.

JEENER, R. (1947). Cytochemical effects of oestradiol. *Nature, Lond.*, **159**, 578.

KREHBIEL, R. H. (1941). The effects of lactation on the implantation of ova of a concurrent pregnancy in the rat. *Anat. Rec.* **81**, 43–65.

LONG, J. A. & EVANS, H. M. (1922). The oestrous cycle in the rat and its associated phenomena. *Mem. Univ. Calif.* **6**, 1–148.

LYON, A. & ALLEN, W. M. (1938). Duration of sensitivity of the endometrium during lactation in the rat. *Amer. J. Physiol.* **122**, 624–626.

MOOG, F. (1946). The physiological significance of the phosphomonoesterases. *Biol. Rev.* **21**, 41–59.

PRITCHARD, J. J. (1948). The distribution of alkaline phosphatase in the pregnant uterus of the rat. *J. Anat., Lond.*, **81**, 352–364.

SELYE, H. & MCKEOWN, T. (1935). Studies on the physiology of the maternal placenta in the rat. *Proc. roy. Soc.* B, **119**, 1–31.

EXPLANATION OF PLATES

All sections illustrated were stained exclusively for alkaline phosphatase. Figs. 1, 2, 5 and 6 are at a magnification of 150. The remainder at a magnification of 1350. In each of these two groups the photographs were obtained under identical conditions of exposure, development, etc. Figs. 7–25 show the uterine epithelium with some of the adjacent stroma at various stages of reproductive life and after castration and the administration of stilboestrol and progesterone.

PLATE 1

Fig. 1. A transverse section of the uterus of a 10-day-old rat.

Fig. 2. A transverse section of the uterus of a 28-day-old rat.

Fig. 3. Part of a uterine gland during oestrus.

Fig. 4. Part of the endometrium during oestrus.

PLATE 2

Fig. 5. Part of the uterine wall and uterine lumen at pro-oestrus.
Fig. 6. Part of the uterine wall and uterine lumen in early oestrus.
Fig. 7. Ten days after birth.
Fig. 8. Twenty-eight days after birth.
Fig. 9. Six weeks after birth.
Fig. 10. Late pro-oestrus, with gross distention.
Fig. 11. Pro-oestrus, with moderate uterine distention.
Fig. 12. Pro-oestrus, before distention is marked.

PLATE 3

Fig. 13. Oestrus—stage of vacuolation.
Fig. 14. Oestrus—stage of leucocytic infiltration.
Fig. 15. Metoestrus.
Fig. 16. Late dioestrus.
Fig. 17. Eighth day of pregnancy.
Fig. 18. Fifteenth day of pregnancy.

PLATE 4

Fig. 19. Pseudopregnancy.
Fig. 20. Eighth day of lactation.
Fig. 21. Eight days after castration.
Fig. 22. Four months after castration.
Fig. 23. Six weeks old, 50γ stilboestrol given 2 days previously.
Fig. 24. Six weeks after castration, 50γ stilboestrol given 2 days previously.
Fig. 25. Five days after pro-oestrus, 5 mg. progesterone given on the 1st and 3rd days.

SOME NEW OBSERVATIONS UPON THE GRANULES OF THE OXYNTIC CELLS OF NORMAL RATS

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INTRODUCTION

Lim & Ma stated in 1926 that the granules of the oxyntic cells of the rat's stomach contained lipoid. They showed that these granules were stained by Janus green B, and they therefore took them to be mitochondria, and, believing that all mitochondria contained lipoid, they presumed that the granules must contain lipoid. They stated, in addition, that the granules could often be impregnated with osmium tetroxide, which they took to be evidence of a lipoid content. The argument rests upon three assumptions, viz. that Janus green B is a specific stain for mitochondria, that all mitochondria contain lipoid, and that osmium tetroxide is a reagent specific for lipoids. It is known, however, that osmium tetroxide may be reduced by substances other than lipoids (Lison, 1936), and even if Janus green B is specific for mitochondria it has not been shown that all mitochondria contain lipoid. It follows that Lim & Ma's observations, though suggestive, do not provide a satisfactory proof of the presence of lipoid in the granules of the oxyntic cells. Recently, Foster in this Department has shown (unpublished work) that the granules of the oxyntic cells of the rat's stomach could be stained with Sudan black, suggesting again the presence of a lipoid. It was therefore decided to try to investigate further the lipoid content of the granules, chiefly by the use of Baker's acid haematein test (1946) for lipines, and by various other methods.

MATERIAL AND METHODS

Twenty-four male Norwegian rats, kept in separate cages, were starved, except for an allowance of water, for 24 hr. They were then killed by coal gas, used in preference to other anaesthetics as being less likely to affect the lipoid content of the cells. The stomach was then removed and pieces of the mucous membrane from the fundus were treated as follows:

(*a*) Fixed in formol-calcium-chloride (Baker, 1944), washed and embedded in gelatin. Sections were then cut at 10μ on the freezing microtome, and coloured with: (i) Sudan black (Baker, 1944), (ii) Sudan II, Sudan III, Sudan IV, Nile blue sulphate.

(*b*) Fixed in formol-calcium-chloride and (iii) Baker's acid haematein test applied (Baker, 1946).

(*c*) Fixed in the following modification of Bouin's fluid:

Saturated aqueous solution of picric acid	50 c.c.
Commercial formalin	10 c.c.
Glacial acetic acid	5 c.c.
Distilled water	35 c.c.

After fixation the lipoids were removed by hot pyridine (Baker, 1946) and the tissues embedded in gelatin. Sections were cut frozen, at 10μ, and treated as follows: (iv) by Baker's acid haematein test, (v) with Sudan black.

(*d*) Lipoids were removed by hot pyridine, after fixation in the modified Bouin's fluid described above. The tissues were then washed and dehydrated, cleared and embedded in wax, and sections cut at 5μ were treated with (vi) Heidenhain's iron haematoxylin.

(*e*) Fixed in Champy's fluid and treated by (vii) the Nassanov-Kolatchew technique, a routine method for the demonstration of the Golgi element by the use of osmium tetroxide.

The following inferences may be drawn from certain reactions to the above methods. A positive reaction to Sudan black, or Sudan II, III or IV, indicates the presence of lipoid, Sudan black frequently demonstrating lipoid when the others fail to do so. A rose or red coloration with Nile blue sulphate, according to Lison (1936), indicates the presence of a non-saturated glyceride. A positive reaction to Baker's acid haematein test shows the presence of lipines and/or several proteins. Hot pyridine, however (as used in the pyridine extraction test), removes lipoids but not the proteins, and thus a subsequent application of the acid haematein test would, if negative, indicate the absence of both the lipoids and the several proteins that react to the test. The combination of a positive reaction to the acid haematein test and a negative reaction following pyridine extraction indicates the presence of lipine alone.

RESULTS

(1) *With Sudan black* (Baker, 1944) (Pl. 1, fig. 1). The granules in the oxyntic cells colour intensely, appearing a bluish black. There are many granules in the cells, some being quite discrete, but the majority are not very sharp in outline. They vary in size, the largest being approximately twice the diameter of the smallest visible, and there are many of intermediate size. There is some clumping of the granules. The nucleus is pale and unstained, but the cytoplasm is grey in patches, indicating the presence of a diffuse lipoid substance. The patchy greyness of the cytoplasm probably accounts for the fact that the majority of the granules do not appear to have a sharp outline. Completely uncoloured areas devoid of granules can frequently be seen marking the position of intracellular canals.

(2) *With Sudan II, III and IV and Nile blue sulphate.* No positive reaction is given by the granules in the oxyntic cells, and there is no indication of a rose or red colour with Nile blue.

(3) *With acid haematein* (Baker, 1946) (Pl. 1, fig. 2). The granules stain black or blue-black. They are numerous and discrete, and more definite than with

Sudan black. The cytoplasm is colourless, and this may account for the discrete appearance of the granules. In positively reacting cells no difference is apparent in the intensity of colouring between those deeply situated and those superficially placed in the tubule, or between cells in one tubule and another, but a few oxyntic cells in the depths of the glands show no positive reaction. It seems unlikely that this is due to a failure of penetration of the reagents, for these cells occur at random, surrounded by positively reacting cells. Except in the negatively reacting cells, there are no unstained refractile granules visible. The granules are dispersed evenly throughout the cytoplasm, save where colourless channels representing the intracellular canals can be seen, and in a few areas there is some clumping of the granules. The granules vary in size within the same cell, the largest having a diameter approximately twice that of the smallest visible. They are all spherical. Some granules are more deeply coloured than others, some appear quite black, some blue-black. It is frequently difficult to tell whether the darkness of the granules is due to clumping and consequent overlapping, or to their size. On the whole the depth of staining is uniform, and where some granules appear more deeply coloured than others this can frequently be shown by careful focusing to be due to the fact that one granule is lying above another.

(4) *With acid haematein following pyridine extraction of lipoids* (Baker, 1946) (Pl. 1, fig. 3). None of the granules in any of the oxyntic cells appears coloured, but they can still be seen by virtue of their refractility. The nucleus stains deeply, as also do the red blood corpuscles (see Baker, 1946).

It may be mentioned here that there are small cells in the connective tissue of the submucosa whose granules are positive to acid haematein and negative after pyridine extraction, although their significance is not known.

(5) *With Sudan black following pyridine extraction of lipoids.* None of the granules of the oxyntic cells is coloured. This is what one would expect, the test being applied merely as a check on the acid haematein test.

(6) *With iron haematoxylin following pyridine extraction of lipoids* (Pl. 1, fig. 4). The granules stain a dark greyish brown and appear in about equal numbers in all cells of similar size, and there appear to be as many granules per cell as in comparable cells to which the acid haematein test has been applied without pyridine extraction.

(7) *With the Nassanov-Kolatchew technique for the Golgi element.* All oxyntic cells show a dense nuclear membrane, and, in addition:

(*a*) The majority show slightly osmiphil granules spread throughout the cytoplasm, and indistinct osmiphil strands in the cytoplasm between the granules.

(*b*) The minority show a definite osmiphil network spreading throughout the cytoplasm. This network, which is slightly more osmiphil than the granules described above, is closely applied round refractile unstained granules.

(*c*) A few cells show a collection of very dark osmiphil strands fairly well localized to one part of the cell.

Many mucous and peptic cells show a typical Golgi element—a localized net between the nucleus and the gland lumen; the oxyntic cells do not.

DISCUSSION

The conclusions arrived at by Lim & Ma (1926) have been confirmed—Sudan black shows that the oxyntic granules contain lipoid. Furthermore, the acid haematein and pyridine extraction tests show that they contain lipine, presumably a phospholipine, since galactolipine is believed to occur only in the central nervous system. Sudan black indicates also the presence of a diffuse lipoid in the cytoplasm.

The application of iron haematoxylin, after the lipoids have been removed by pyridine, shows that a stainable residue is present, probably protein or partly protein in nature. It can therefore be inferred that the granules are of a double nature, partly lipoid and partly non-lipoid. It is probable that the lipoid forms an envelope to a non-lipoid core, though the evidence is not yet adequate to decide this point.

There are other cell inclusions having lipoid and non-lipoid components, namely, (1) mitochondria (Claude, 1941; Bourne, 1942; Hoerr, 1943; Baker, 1946), and (2) the Golgi element (Hirsch, 1939; Baker, 1944; Foster, 1947).

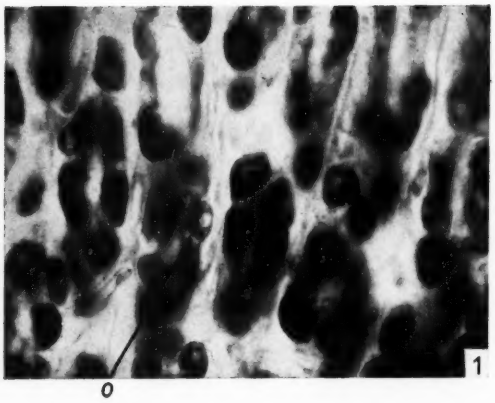
Kölster in 1913 stated that there were two types of Golgi element in the oxyntic cell, one completely filling the cell, the other being more localized. Both Siraska (1929) and Shiraska (1930) denied the existence of Golgi material in this cell. In 1932 Beams & King investigated the Golgi element in the oxyntic cell of the rat, and although they never found as complete a network as reported by Kölster, they described it as being present in the form of robust filaments scattered throughout the cytoplasm. In my preparations using the Nassanov-Kolatchew method, both types seen by Kölster are undoubtedly present, together with a third in which the Golgi element is seen as black granules with osmiphil strands in the cytoplasm between them. I would suggest that the dissimilarities in the three types of Golgi element seen are due to different functional states of the cells concerned.

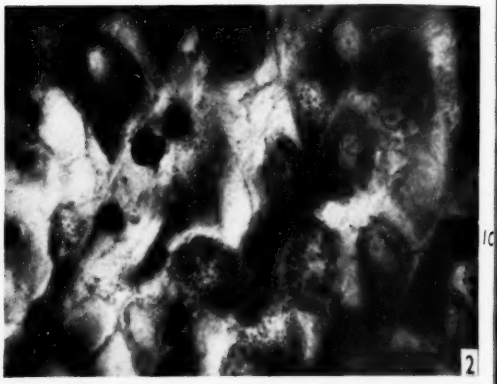
A connexion may be seen here between the Golgi element, as shown in the preparations mentioned above, and those parts of the oxyntic granules proved to be lipoid. For, supposing that the lipoid in all the granules represented the osmiphil part of the Golgi element, then one would expect to find a very large Golgi element, or a continuous net, occupying the greater part of the cell; and such is often the case. Where, as is sometimes observed, the Golgi element occupies only a small proportion of the cell, then, on the hypothesis just suggested, this could mean that a smaller number of the lipoid-containing granules reduced the osmium tetroxide, and perhaps it is these that are observed in some cells to be more deeply coloured with acid haematein than the rest.

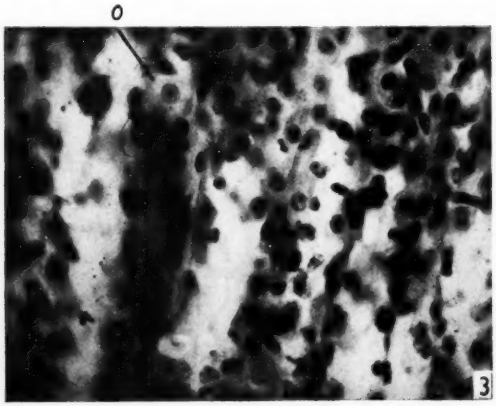
SUMMARY

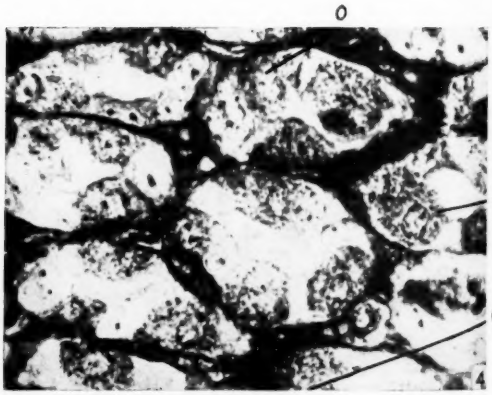
1. The granules of the oxyntic cell of the rat's stomach contain lipoid, as shown by their intense colouring with Sudan black.

2. They contain lipine, probably phospholipine, demonstrated by a positive reaction to Baker's acid haematein test, and a negative reaction to this test following the removal of lipoids by hot pyridine.









GORDON MENZIES—THE GRANULES OF THE OXYNTIC CELLS OF NORMAL RATS

3. They are of a double nature, possessing a core that is neither lipine nor lipoid, since they are stainable by iron haematoxylin after the lipoid component has been removed.

4. There is some evidence that the cytoplasm of the oxyntic cell contains a diffuse non-lipine lipoid material. It is indicated by Sudan black.

I am greatly indebted to Dr C. L. Foster, who suggested this line of research to me, and I wish to express my thanks to him and to Prof. F. Goldby, for their continued interest and help in this work.

REFERENCES

BAKER, JOHN R. (1944). The structure and chemical composition of the Golgi element. *Quart. J micr. Sci.* **85**, 1–72.

BAKER, JOHN R. (1946). The histochemical recognition of lipine. *Quart. J. micr. Sci.* **87**, 441–470.

BEAMS, H. W. & KING, R. L. (1932). Notes on the cytology of the parietal cells of the stomach of the rat. *Anat. Rec.* **53**, 31–41.

BOURNE, G. (1942). *Cytology and Cell Physiology*, ed. Bourne. Oxford: Clarendon Press.

CLAUDE, A. (1941). *Cold Spr. Harb. Symp. Quant. Biol.* **8**, 144–153.

FOSTER, C. L. (1947). Some observations upon the Golgi elements of the anterior pituitary cells of normal and stilboestrol-treated male rats, using Sudan black technique. *Quart. J. micr. Sci.* **88**, 409–418.

HIRSCH, G. C. (1939). Form und Stoffwechsel der Golgi-Körper. *Protoplasma Monographs*. Berlin.

HOERR, N. L. (1943). Methods of isolation of morphological constituents of the liver cell. *Biol. Symp.* **10**, 185–231.

KÖLSTER, R. (1913). Ueber die durch Golgis Arsenik- und Cajals Urannitrat-Silbermethode darstellbaren Zellstrukturen. *Anat. Anz., Verh. anat. Ges. Jena.* **44**, 124–132.

LIM, R. K. S. & MA, W. C. (1926). Mitochondrial changes in the cells of the gastric glands in relation to activity. *Quart. J. exp. Physiol.* **16**, 87–110.

LISON, L. (1936). *Histochimie Animale*. Paris: Guathier-Villars.

SHIRASKA, M. (1930). Studien über die morphologischen Veränderungen des Golgischen Apparatus in den Zellen des Verdauungssystems des Kaninchens infolge der Darreichung von K-, Ca- und Mg-Salzen. *Folia anat. japon.* **8** (quoted from Beams & King, 1932).

SIRASKA, S. (1929). The existence or the non-existence of the Golgi network in delomorphous cells of the gastric glands (in Japanese). *Nippon Shokwakibyogakukwai Zasshi*, **28** (quoted from Beams & King, 1932).

EXPLANATION OF PLATE

Fig. 1. Fundic area of rat's stomach coloured with Sudan black, ×500.

Fig. 2. Granules in oxyntic cells, after application of Baker's acid haematein test, ×1050.

Fig. 3. Absence of colouring of granules when Baker's acid haematein test is applied following pyridine extraction of lipoid, ×500. N.B. The oxyntic nuclei are now coloured (see Baker, 1946).

Fig. 4. Appearance of oxyntic granules after staining with Heidenhain's iron haematoxylin, following pyridine extraction according to Baker's method, ×950.

O = oxyntic cell. *IC* = site of an intracellular canal. Figs. 1–3 are cut along the tubules of the fundic glands. Fig. 4 is cut across the tubules.

A NEW HISTOCHEMICAL METHOD FOR GLYCOGEN

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The method to be described is based first on Bauer's (1933) observation that glycogen, after treatment with chromic acid, is rendered insoluble in water and exhibits the reactions for aldehydes; and secondly on Mitchell & Wislocki's (1944) observation that under certain conditions glycogen will reduce ammonium-silver compounds. The following method is the outcome of experiments made to determine whether the specificity of Bauer's method could be associated with the sharpness of definition and ease of photographic reproduction of Mitchell & Wislocki's method, and it was found that glycogen in tissue sections was capable of reducing ammonium-silver compounds specifically after treatment with chromic acid. Exceptionally clear and detailed pictures of glycogen distribution in a number of tissues were obtained.

METHOD

Tissues are fixed in ice-cold absolute alcohol for 24–48 hr. and then embedded in paraffin in the usual way. Sections are cut and flattened on 70 % alcohol and dried on the slide without the use of adhesives. After removal of wax they are coated with celloidin by immersion in 0·5 % solution of celloidin in alcohol-ether followed by hardening in 70 % alcohol.

After passing to distilled water, the sections are placed in 4 % chromic acid at 55° C. for 25–30 min. and then washed well in distilled water. They are transferred to Foot's silver diamino hydroxide solution for 2–5 min. and then, without washing, immersed in Wilder's formol-uranium reducing solution, with agitation, for 2 min.

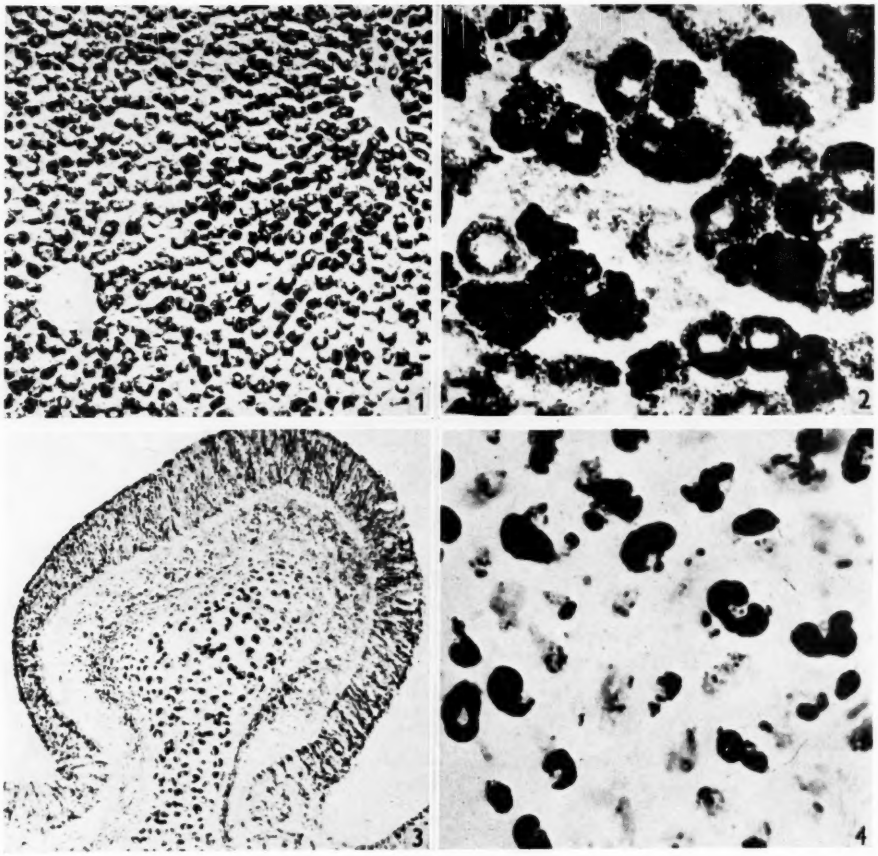
They are again washed in distilled water and toned for 2–5 min. in 0·2 % gold chloride solution, rinsed in tap water and placed in 5 % sodium thiosulphate solution for 2 min.

After a further wash in distilled water the sections may be counter-stained (light green or acid fuchsin are suitable) and mounted.

Glycogen is stained purplish black by this method, and the background is clear. No other tissue components take the stain.

That the granules stained by this method consisted of glycogen was confirmed by the saliva test, which is based on the fact that glycogen is the only polysaccharide present in animal tissues which is digested by salivary ptyalin. After the sections had been incubated for 30 min. in a 25 % solution of saliva in tap water at 37° C. no reduction of silver occurred after subsequent treatment with chromic acid and silver diamino hydroxide.

Pl. 1, figs. 1–4 illustrate examples of the results obtained.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DISCUSSION

The method described differs from that of Bauer (1933) in the substitution of silver diamino hydroxide for sulphuretted basic fuchsin (Feulgen's reagent) as the means of visualizing glycogen after treatment with chromic acid. This has the advantage that the precipitate of reduced silver is more sharply defined, particularly in relation to very small particles of glycogen, than the basic fuchsin stain; it can also be photographed much more easily and with better contrast.

Mitchell & Wislocki's (1944) silver reduction method for glycogen is much more laborious and lengthy, and it has the disadvantage that it is not specific for glycogen, as reticular fibres are heavily impregnated.

SUMMARY

A new method for the histochemical detection of glycogen is described, based on the reduction of silver diamino hydroxide by glycogen after preliminary treatment with chromic acid.

REFERENCES

Bauer, H. (1933). Mikroscopisch-chemischer Nachweis von Glykogen und einigen anderen Polysacchariden. *Z. mikr.-anat. Forsch.* **33**, 143–160.

Mitchell, A. J. & Wislocki, G. B. (1944). Selective staining of glycogen by ammoniacal silver nitrate: a new method. *Anat. Rec.* **90**, 261–266.

EXPLANATION OF PLATE

Fig. 1. Section of liver from an adult rat. ×150.
Fig. 2. Section of liver from an adult rat. ×600.
Fig. 3. Section of nasal concha from a 20-day rat embryo. ×150.
Fig. 4. Section of cartilage from a nasal concha of a 20-day rat embryo. ×600.

THE EFFECT OF PERIPHERAL CONNEXIONS ON THE MATURATION OF REGENERATING NERVE FIBRES

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INTRODUCTION

Previous work (Sanders & Young, 1944, 1946; Weiss & Taylor, 1944; Weiss, Edds & Cavanaugh, 1945; Aitken, Sharman & Young, 1947) has shown the importance of the effect which the peripheral connexions have on regenerating nerve fibres. The present work was planned to examine in detail some of the factors which might contribute to this effect.

Answers were sought to the following questions: (*a*) Does the distance travelled by regenerating fibres modify the process of regeneration? (*b*) Do fibres turn at the cut end of a nerve and grow back in a central direction? (*c*) To what extent does close contact of regenerating fibres with muscle tissue influence maturation?

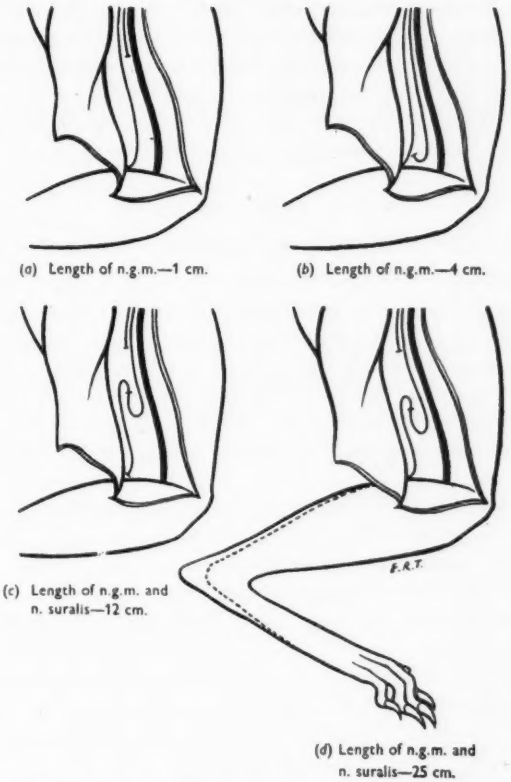
MATERIALS AND METHODS

Adult rabbits of different breeds were used, and the nerve to the medial head of the gastrocnemius muscle (n.g.m.) was selected, as in the previous work (Aitken *et al.* 1947). In all cases the n.g.m. was crushed as high as possible, with fine smooth watch-maker's forceps. In order to study the behaviour of new fibres growing down various lengths of nerve but not reaching the end organ, the nerve was also cut distally and the peripheral stump avulsed from the muscle. In this way a neuroma was caused to form at distances 1–4 cm. from the crushed point. In order to study regeneration over still longer lengths the proximal cut end of the n.g.m. was joined to the distal end of the severed sural nerve, which lies near by, and in this way the length of the regenerating pathway could be increased up to about 25 cm. in large rabbits. The union was maintained by means of coagulated fibrin. In some animals the sural nerve was again cut below the knee (see Text-fig. 1).

In this series, regeneration was allowed to proceed for 100 days, and the nerves were then fixed in order to determine the diameter of the new fibres at a level 1·5 cm. below the crushed point.

In another series of animals, to determine the effect of the muscle on regeneration, the n.g.m., after being crushed as high as possible and cut distally, was implanted into a nearby muscle. Biceps femoris was used and the implantation was made near the nutrient artery, by passing the end of n.g.m. along the track in the muscle made by carefully inserting a round-bodied needle between the fasciculi. This manipulation was carried out with the minimum amount of trauma to avoid damaging the muscle fibres. The implant was held in position with coagulated fibrin. The functional state of biceps femoris was varied by removal of the entering nerves from the sciatic trunk on one side, using the opposite side as a control with a normally innervated biceps muscle. In an attempt to prevent reinnervation, the cut ends of the nerve to biceps femoris were formalinized in some animals, but the method was abandoned owing to the danger of injury to the main sciatic trunk.

All animals were examined after a 100-day period and the n.g.m. was identified. In most cases the nerve was stimulated with an induction coil and any muscle response noted. Occasionally, the implantation was not maintained in the muscle and in these cases the nerve formed a neuroma on the surface of biceps or in the fascia.



Text-fig. 1. Diagram showing the four types of experiment to determine the effect of the distance travelled by regenerating fibres on the maturation of the nerves. (*a*) and (*b*), lengths of 1 and 4 cm. from n.g.m.; (*c*) and (*d*), lengths of 12 and 25 cm. from n.g.m. and n. suralis.

Where the implant was undisturbed, the minimum stimulus needed to evoke a muscle response was noted with the electrodes placed about 2 cm. from the site of the implantation. As far as possible, the stimulation of the nerves was standardized and in each case, after the threshold with the nerve intact had been noted, the nerve was then cut centrally, isolating it from the central nervous system and finally crushed close to the muscle to show whether the contraction was due to spread of the stimulus along the nerve.

In all the different experiments the nerves were fixed in the following modification of Flemming's solution (2 % osmic acid in distilled water, 4 c.c.; 1 % chromic acid

in distilled water, 15 c.c.; glacial acetic acid, 1 drop) for not more than 24 hr., dehydrated without preliminary washing, cleared in cedar-wood oil and embedded in paraffin wax after an intermediate stage in cedar-wood oil-benzol-paraffin wax mixture. Sections, 5μ thick, were cut at comparable levels in all nerves. In the shortest lengths of n.g.m. the sections were cut as near as possible to the neuroma, but in all others at a point about 1·5 cm. below the crush. The site of the crush was determined by a slight swelling in the nerve, by the presence of a marker (indian ink or black silk) or by the central cut end of the sural nerve which was found usually close to the crushed point. The sections were stained by Weigert's method and photographed at 750× magnification on to bromide paper. Differential counts were made of the fibre diameters which were classified into 2μ groups.

RESULTS

A. *Effect of length of regeneration path on the degree of maturation*

The experiments provided data for the following lengths of the regenerating path: (*a*) regeneration along 1 cm. of peripheral path, n.g.m.; (*b*) regeneration along 4 cm. of peripheral path, n.g.m.; (*c*) regeneration along 12 cm. of peripheral path, n.g.m. and n. suralis; (*d*) regeneration along 25 cm. of peripheral path, n.g.m. and n.suralis.

The differential counts of the fibre sizes just below the crush obtained from the nerves in these experiments are given in Table 1 along with the total number of fibres in the nerve, the mean diameter for the fibres over 6μ in diameter and their number.

Table 1 (*a*) shows that in the short peripheral path the total number of fibres is large and usually over 2000. The great majority of these fibres are small and poorly myelinated, and the mean diameter of the groups over 6μ is 7·33μ. As the neuroma in these experiments is very near to the crush there will be a large number of fibres which turn at the neuroma and pass back along the nerve. They will have been counted twice (see later).

Table 1 (*b*) contains the results obtained from those nerves where the distance travelled by the regenerating fibres was 4 cm. of n.g.m. There is a diminution in the total number of fibres, with an increase in the number of those over 6μ in diameter, the mean of the latter being 7·68μ.

Table 1 (*c*) and (*d*) show a continuation of this trend, the total number falls with an increase in the over 6μ groups and an increase in the mean diameter to 7·98μ in the 12 cm. pathway and 8·74μ in the 25 cm. pathway.

After an examination of Table 1, it was decided to consider only the larger fibre (over 6μ) groups and the mean fibre diameter of those fibres was taken as an index of maturation in the nerve. This was done in an attempt to prevent the number of small fibres (some of which would be counted twice) masking the effect of any change in the number and distribution of the larger fibres. From each set of comparable experiments a series of mean diameters was obtained and considered as a sample of nerves regenerating for the given length. Is there any simple relationship between the degree of maturation and the length of the regeneration path? Inspection of the figures in Table 1 suggests a high degree of correlation between maturation and length regenerated, but the estimation of the correlation coefficient would only give

the extent of the connexion between the two variables. It would not give any information as to the degree in which change in length affects maturation.

If, however, the mean maturation estimations for all the nerves are plotted against the length regenerated, it appears that there is some reason to anticipate a linear relationship between the two variables. In more statistical terminology we may reasonably expect a significant linear regression of maturation on length of the regeneration pathway. This line can be computed and is shown in the diagram (Text-fig. 2), indicating that over the range of the experiments there is an increase

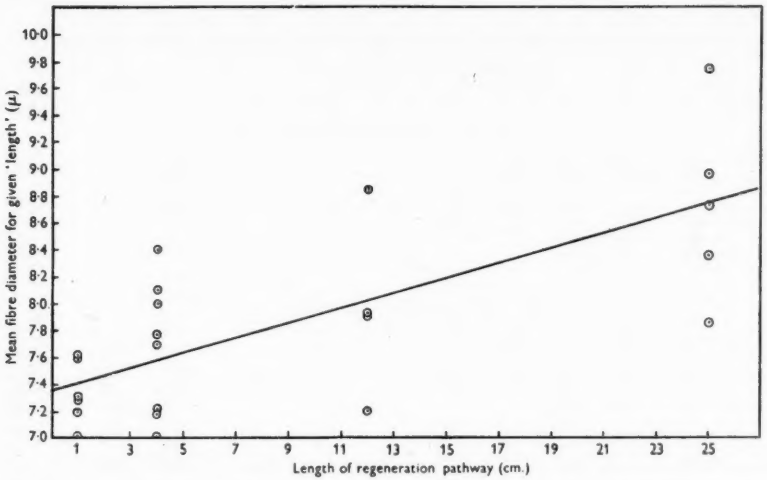
Table 1. *Distribution of fibres in regenerating nerves of different lengths*

Specimen	Diameter (μ) 0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	Total	No. >6μ	Mean of >6μ fibres
					(*a*) N.g.m. with 1 cm. peripheral path						
48 (*a*)	326	1254	381	91	8	—	—	—	2060	99	7·32
51 (*f*)	96	1528	558	207	66	8	—	—	2463	281	7·58
56 (*f*)	311	2513	521	198	81	2	—	—	3625	281	7·60
57 (*f*)	378	1576	409	199	22	—	—	—	2584	221	7·20
58 (*c*)	216	1115	363	150	27	—	—	—	1871	177	7·31
59 (*a*)	315	1750	375	23	—	—	—	—	2463	23	7·00
									Mean of group		7·33
					(*b*) N.g.m. with 4 cm. peripheral path						
29 (*b*)	132	724	318	124	41	9	3	—	1351	177	7·77
51 (*a*)	95	764	349	102	10	—	—	—	1320	112	7·18
55 (*a*)	41	540	193	125	115	41	—	—	1055	281	8·40
56 (*a*)	35	560	166	162	107	18	—	—	1048	287	8·00
57 (*c*)	85	711	311	122	3	—	—	—	1222	125	7·05
58 (*a*)	186	1058	368	128	16	—	—	—	1756	144	7·22
59 (*b*)	45	583	194	124	117	11	—	—	1074	252	8·10
185 (*b*)	36	518	263	138	67	3	—	—	1025	208	7·70
									Mean of group		7·68
					(*c*) N.g.m. joined to sural nerve—total length 12 cm.						
83 (*a*)	35	414	172	112	160	73	6	—	972	351	8·85
89 (*a*)	53	589	279	119	68	11	—	—	1199	198	7·91
95 (*a*)	61	503	117	143	110	5	—	—	939	258	7·93
105 (*b*)	124	322	270	233	5	9	1	—	964	248	7·21
									Mean of group		7·98
					(*d*) N.g.m. joined to sural nerve—total length 25 cm.						
89 (*c*)	20	589	277	144	114	46	—	—	1190	304	8·36
539 (*d*)	55	274	108	94	120	58	—	—	709	272	8·74
539 (*a*)	14	160	110	119	129	104	5	—	641	357	8·97
541 (*a*)	56	444	225	152	93	8	—	—	978	253	7·86
546 (*a*)	23	192	83	86	106	94	53	5	642	344	9·75
									Mean of group		8·74

of 0·5 μ in the mean diameter of the larger regenerating fibres in each 10 cm. travelled. That the effect is a real one and not due to chance is shown by the regression coefficient which is significant on the 0·001 level ($t = 4·7$, $f = 21$).

These experiments therefore definitely indicate that regenerating fibres which grow for a greater distance become larger. Nothing very definite can be said about the form of the relationship, the diameter more nearly follows the length than its logarithm, but other possibilities are not excluded. It must also be remembered that the very longest fibres make endings different from the others (in the skin rather than in a neuroma).

As the length of the peripheral path and the rate of progress of the nerve fibres down the path can be estimated, it is possible to calculate the time taken to reach the end of the Schwann tubes, and by deducting this figure from 100 to determine the time allowed for maturation. Allowing 5 days for the fibres to cross the crush, 5 days to cross the union and a growth rate of 5 mm. per day above the union but of only 3 mm. a day below the union (Gutmann, Guttman, Medawar & Young, 1942) we obtain the results summarized in Table 2.



Text-fig. 2. Linear regression of degree of maturation on the length travelled by the regenerating nerves, maturation being measured as the mean diameter of the larger ($> 6\mu$ in diameter) fibres.

Table 2. *The estimated times allowed for maturation of the regenerating fibres of n.g.m. after travelling different distances*

Distance travelled (mm.)	Estimated time to reach end of nerve (days)	Time allowed for maturation (days)
10	7	93
40	13	87
120	45	55
250	88	12

In the longest nerves many of the fibres would be actively growing until 12 days before the biopsy. The specimens with neuromas at the cut end of the n. suralis below the knee (12 cm.) would have 55 days in which to mature, yet the mean diameter of the fibres is less than that found in the longest nerves (25 cm.) (see Text-fig. 1).

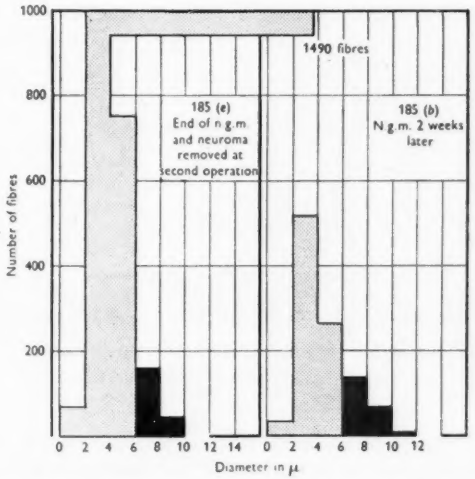
B. *Turning back of regenerating fibres at a neuroma*

When regenerating nerve fibres reach the end of the Schwann tubes, without making contact with a peripheral end organ, a neuroma is formed. It has been suggested by Weiss *et al.* (1945) that some of the fibres turn and re-enter the Schwann tubes and travel in a central direction. This phenomenon was clearly demonstrated

in one of the experiments where the nerve was crushed, cut lower down, allowed to regenerate and form a neuroma, and was then operated on again, the neuroma being cut off 2 weeks before the terminal biopsy. Comparison of the two specimens 185 (*e*) and 185 (*b*) in Table 3 shows that the total number of fibres had been reduced to one-half.

Table 3 (*a*) and (*b*). *Counts of regenerating fibres in the n.g.m. immediately proximal to a neuroma and in the same nerve 2 weeks after the neuroma had been removed*

Specimen	Diameter (μ) 0–2	2–4	4–6	6–8	8–10	10–12	Total
	(*a*) Portion of n.g.m. with neuroma attached						
185 (*e*)	68	1490	750	158	42	—	2508
	(*b*) N.g.m. 2 weeks after removal of neuroma						
185 (*b*)	36	518	263	138	67	3	1025



Text-fig. 3. Histograms of the distributions of fibre sizes in n.g.m. immediately proximal to a neuroma and in the same nerve two weeks after the neuroma had been removed.

Unfortunately, owing to the nearness to the neuroma, it was impossible to obtain a section of which all parts could be counted. However, a reliable estimate was made by determining the total area of the photograph with a planimeter (727 sq.cm.) and calculating the number of fibres in the size groups from the area which was capable of being counted (426 sq.cm.).

Examination of the figures in Table 3 shows that the greater differences are to be found in the groups of smaller fibres. From these findings it does seem that a great number of small regenerating fibres returns along the nerve trunk and this suggests that the larger fibres are the better index of maturation as the number of larger fibres was almost the same in the two specimens.

Text-fig. 3 shows the histograms of these two specimens, and Pl. 1, figs. 1 and 2 the photographs of the cross-sections which were counted. The difference in size of the sections is due to the proximity to the neuroma and the number of fibres.

C. *Effect of muscle state on maturation of regenerating fibres*

On examination of the animals after 100 days, it was found that the biceps muscle was not greatly wasted on the paralysed side, though stimulation of the sciatic nerve high in the thigh produced no contraction of the muscle. Stimulation of n.g.m. gave rise to a contraction of a bundle of muscle fibres. By increasing the strength of the stimulus it was possible to make more muscle fibres respond, though never did the muscle react as when the nerve to the normal biceps on the other side was stimulated. The paralysed muscles responded at a higher threshold of stimulation of the nerve than did the normal muscles. The differential counts of the fibre populations of these nerves are given in Table 4.

Table 4. *Distribution of fibres in regenerating n.g.m. when implanted into normal and paralysed biceps femoris muscles*

Specimen	Diameter (μ) 0–2	2–4	4–6	6–8	8–10	10–12	12–14	Total	No. $>6\mu$	Root mean sq. diameter of fibres $>6\mu$ (D)
					(*a*) Implantation into normal biceps					
417 (*r*)	52	1035	256	177	23	—	—	1790	200	7·2
428 (*r*)	115	716	287	165	67	8	—	1358	240	7·9
443 (*c*)	175	586	194	163	81	—	—	1199	244	7·7
483 (*a*)	316	973	419	77	216	2	—	2003	295	6·3
642 (*c*)	12	588	375	228	66	10	—	1279	304	7·4
643 (*b*)	55	426	193	189	78	1	2	944	270	7·7
					(*b*) Implantation into paralysed biceps					
417 (*l*)	6	817	394	326	160	97	11	1811	594	8·5
428 (*l*)	85	730	285	150	29	13	—	1292	192	7·7
443 (*a*)	151	612	183	134	66	9	—	1155	209	7·9
483 (*d*)	138	968	185	85	111	69	49	1605	314	9·7
642 (*a*)	13	347	133	110	105	65	30	803	310	9·3
643 (*c*)	44	416	216	136	98	36	5	951	275	8·5

The larger fibres ($>6\mu$) were again used as an indication of the degree of maturation. The hypothesis that the distributions of the larger-sized fibres are the same whether the nerve terminates in a normal or paralysed muscle was made and calculations gave the following results:

Animal	X^2	f	$P(X)^2$
417	50·84	1	0·001
428	11·757	2	0·01
443	0·36	1	0·6
483	13·91	2	0·001
642	118·86	2	0·0001
643	21·8	2	0·001

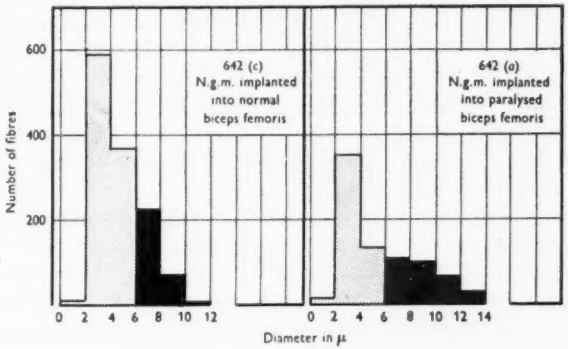
Except in animal 443, the hypothesis is rejected in every case. When the total X^2 is considered, $X^2=217{\cdot}53$, $f=10$ and $P(X)^2=0{\cdot}001$.

The possibility of these samples belonging to the same population is therefore exceedingly remote.

When the root mean square diameters (D) of the fibres more than 6μ in diameter

are considered it is evident that the nerves which regenerate into the paralysed muscles are larger in all experiments except one (428).

Pl. 1, figs. 3 and 4 show photographs of sections of nerves (642*a* and 642*c*) which have been implanted into normal and paralysed muscles. It will be noticed that the former produces a condition in which the Schwann tubes are filled with many small fibres whereas, in the latter, the tubes contain fewer fibres and usually one large fibre. Histograms of the same two nerves (Text-fig. 4) demonstrate the reduction in the total number of fibres—especially the smaller groups—and an increase in the larger fibre groups.



Text-fig. 4. Histograms of the distributions of fibre sizes in regenerating n.g.m. after implantation into normal or paralysed biceps femoris.

DISCUSSION

From the preceding results, it is evident that the distance travelled by regenerating nerve fibres and the possibility of making a functional connexion with muscle both influence in a marked degree the process of maturation.

A. *Effect of length*

The diameters of normal nerve fibres are usually taken to vary according to the type of functional activity of the fibres (Erlanger & Gasser, 1937). In these experiments, the regenerating fibres rarely attain a size more than 12–14μ in diameter, they are non-functional and therefore the peripheral connexion can have no stimulating effect on the maturation processes. Where the n.g.m. was united with the n. suralis, certain new factors were introduced which must be considered. The union of the nerves, the smaller Schwann tubes of the sensory nerve and the presence of peripheral end-organs in the skin are the most important factors. The unions were made with no tension between the two nerves and the positions were maintained with coagulated plasma. At biopsy, the unions were examined and found to be intact and sections of the n. suralis proved that many fibres had passed down into this nerve. Pl. 1, fig. 5 is a photograph of a longitudinal section of one of these unions (95*d*). There is always the possibility, however, that a few fibres escaped at the site of the union into the surrounding fascia or even turned back up the n.g.m.

The smaller Schwann tubes might tend to impede the flow of axoplasm and so increase the diameter of the fibres above the union.

In the normal n.g.m. there are a number of sensory fibres which might find a pathway towards an end organ in the skin. These fibres were presumably non-functional, as stimulation of the skin supplied by n. suralis evoked no response, and when the n. suralis was electrically stimulated no withdrawal of the leg occurred.

The effect of the length of nerve regenerated was investigated by Sanders & Young (1946), who considered that the process of maturation was independent of it. They employed varying lengths of the large peroneal nerve and joined it to the still larger tibial nerve. Both of these nerves have considerable mixed muscle and sensory (skin) components and the regenerating fibres would eventually make contact with end organs, many of them on the muscle fibres. Though the two sides would be partly comparable, no indication is given of the possibility of the motor end-plate connexion masking the length effect. The difference in length was 55 mm., which according to the present findings would account for a shift upwards of $0{\cdot}25\mu$ in the mean diameter of the fibres, a figure which could almost certainly be accounted for by the varying peripheral connexions of the fibres.

Time for maturation. It has been suggested by Weiss *et al.* (1945) that the nerve fibre increases in diameter after the growing end comes to rest. In the present series of experiments, those nerves which could regenerate along the terminal branches of n. suralis into the foot would have only 10–20 days in which to mature, whereas those nerves which ended in neuromas in the thigh or leg would have 70 or 50 days. The degree of maturation, however, bore an inverse relationship to the time. In spite of the long time which could be used for maturation, the shorter lengths had nerves of a smaller mean diameter than the longer nerves. Previous work (Aitken *et al.* 1947) has shown that when nerves are allowed to regenerate and form neuromas for periods up to 200 days, though the number of fibres is reduced in the longer periods, there is no increase in the diameter of the fibres. It is therefore probable that in considering the degree of maturation the distance travelled is a more important factor than the time.

B. *Effect of overcrowding*

The effect of overcrowding the Schwann tubes by a large number of small fibres is most pronounced in the shorter lengths of nerve. Their numbers over the total series of nerves vary considerably and for no very obvious reason, even some of the implants into paralysed muscle (Table 4 (*b*)) having a total of over 1000 fibres in the groups under 6μ in diameter. The larger-sized fibres seem to form a more constant and stable series, and in the nerve which had the neuroma removed 2 weeks before biopsy there was a difference of only 8 between the number of large fibres in the specimen of nerve with the neuroma and that found in the nerve itself. It is realized that there is a difficulty in making a definite statement concerning this matter as sections cut near the neuroma are very difficult to count accurately owing to the obliquity of many of the fibres.

When Pl. 1, figs. 1 and 2 are compared, it is seen that many of the Schwann tubes in fig. 2 (section of n.g.m. after removal of the neuroma) contain only one fibre and often this fibre is small. During the 14 days since operation, many of the small fibres have degenerated, but the remainder do not seem to have increased in size.

C. *Effect of muscle state*

It has long been maintained that the nerve supply of mammalian muscle is such that each muscle fibre has one motor end-plate (Wilkinson, 1929; Denny-Brown & Pennybacker, 1938), though some workers (Aghduhr, 1916; Cuajunca, 1932) have reported the presence of multiple endings on a fibre. In normally innervated muscle there will therefore be a state of equilibrium between muscle and nerve, and when a 'foreign' regenerating nerve is made to grow into a fully innervated muscle, it will lie in contact with muscle fibres which are already innervated. Yet when the nerve is examined the process of maturation is found to have proceeded farther than in a nerve of comparable length which forms a neuroma in the fascia (compare results in Tables 1 (*b*) and 4 (*a*)). The close proximity to muscle tissues does thus seem to have an effect on the process of maturation. Examination of nerves in normal muscles which is being carried out shows that many fine fibres pass between the muscle fibres with little or no attempt to form motor end-plates.

When a regenerating nerve is implanted into a paralysed muscle the process of maturation is greatly facilitated (Table 4 (*b*)); not only is the number of small fibres usually reduced but the larger fibres are significantly increased. When these results are compared with those previously reported (Aitken *et al.* 1947) for maturation in regenerating nerves following union with a muscle nerve, it is found that the maturation after implantation is poorer than after union. In the latter case, most of the motor end-plates were reinnervated and the whole muscle contracted on stimulation. Stimulation of an implanted nerve in a paralysed muscle gave rise to contraction of a few bundles of muscle fibres. Histological examination of the paralysed muscle revealed no innervated 'native' motor end-plate but a very different neuroma from that which was found in a normal muscle. It would seem that the implant responds to the demand on the part of the paralysed muscle and that the most potent stimulus to maturation is the opportunity to form new motor end-plates. As counts of the new motor end-plates were not made, it is impossible to correlate the degree of maturation with the number of functioning muscle units but the number of endings was never very great and they were mostly close to the implant.

The growing ends of the nerve fibres would reach the muscle in about 13 days after the operation and they would have 87 days in which to ramify amongst the muscle fibres. The results of electrical stimulation showed that the functional spread of the nerves was restricted. This was specially marked in the experiments where the implantation was made into a normal muscle.

Fort (1938), working with Weiss, has studied some of the factors involved in the establishment of neuro-muscular connexions in toads. He suggests that denervated muscle may be more 'permeable', though he admits that his evidence is inconclusive. Attempts to arrest the process of reinnervation in a denervated muscle by use of a Ringer extract of normal muscle were also not effective.

From the present work it would seem that the juxtaposition of regenerating nerve fibres and muscle fibres is sufficient to initiate the process of maturation, but that the opportunity of forming a functional motor end-plate on muscle fibres is a very strong stimulus to continuation of the process.

SUMMARY

1. Experiments in which nerves were allowed to regenerate along pathways of different lengths (1, 4, 12 cm. ending in a neuroma, and 25 cm. ending in the skin) showed that the longest nerves had largest diameters. Over the range investigated there was an increase of 0·5μ in the mean diameter of the fibres for each 10 cm. travelled.

2. Almost half the regenerating fibres turn round at a neuroma and travel back along the nerve. This effect is greatest in those cases where the regeneration path is short.

3. Maturation of a regenerating nerve is much more complete when the nerve fibres are allowed to make contact with paralysed (denervated) muscle fibres than when the nerves grow into a normal muscle or into fascia. The possibility of making new functional motor end-plates or reinnervating those which have been denervated produces a marked increase in the degree of maturation.

The author wishes to thank Prof. J. Z. Young for his interest and encouragement with this work, Mr D. Sholl for the statistical analyses, Mr L. Hanning for technical assistance.

REFERENCES

Agduhr, E. (1916). Morphologischer Beweis der doppelten (plurisegmentalen) motorischen Innervation der einzelen quergestreiften Muskelfascern bei den Säugetieren. *Anat. Anz.* **49**, 1–13.

Aitken, J. T., Sharman, M. & Young, J. Z. (1947). Maturation of regenerating nerve fibres with various peripheral connexions. *J. Anat., Lond.*, **81**, 1–22.

Cuajunca, F. (1932). The innervation of the neuromuscular spindles. *J. comp. Neurol.* **54**, 205–226.

Denny-Brown, D. & Pennybacker, J. B. (1938). Fibrillation and fasciculation in voluntary muscle. *Brain*, **61**, 311–334.

Erlanger, J. & Gasser, H. S. (1937). *Electrical Signs of Nervous Activity*. University of Pennsylvania Press.

Fort, W. B. (1938). An experimental study of the factors involved in the establishment of neuromuscular connexions. University of Chicago Libraries (Thesis for Ph.D.).

Gutmann, E., Guttmann, L., Medawar, P. & Young, J. Z. (1942). The rate of regeneration of nerve. *J. exp. Biol.* **19**, 14–44.

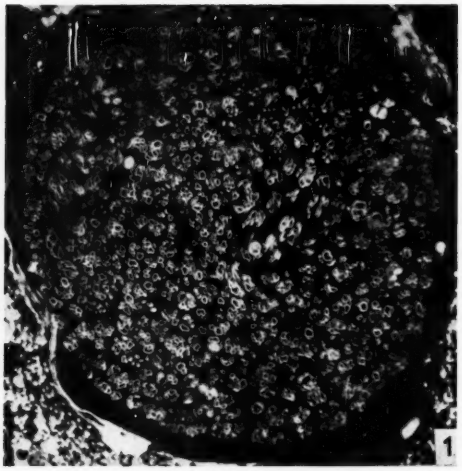
Sanders, F. K. & Young, J. Z. (1944). The role of the peripheral stump in the control of fibre diameter in regenerating nerves. *J. Physiol.* **103**, 119–136.

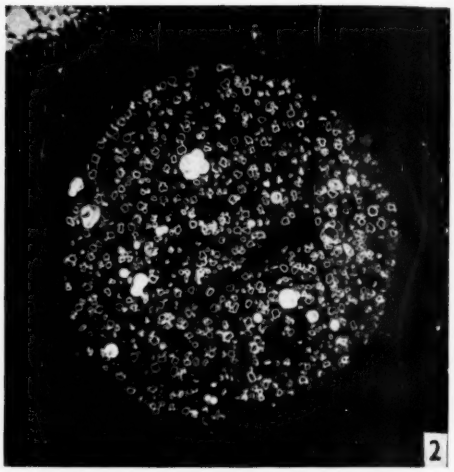
Sanders, F. K. & Young, J. Z. (1946). The influence of peripheral connexion on the diameter of regenerating nerve fibres. *J. exp. Biol.* **22**, 203–212.

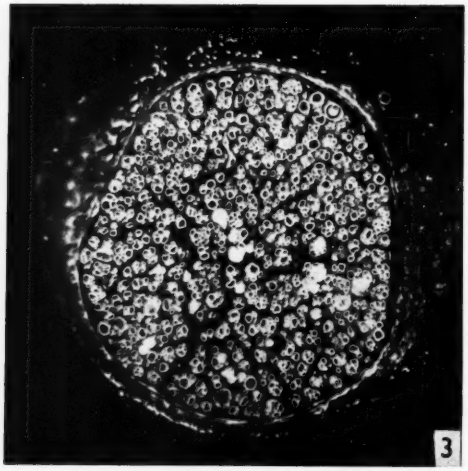
Weiss, P., Edds, McV. Jnr. & Cavanaugh, M. (1945). The effect of terminal connexions on the calibre of nerve fibres. *Anat. Rec.* **92**, 215–233.

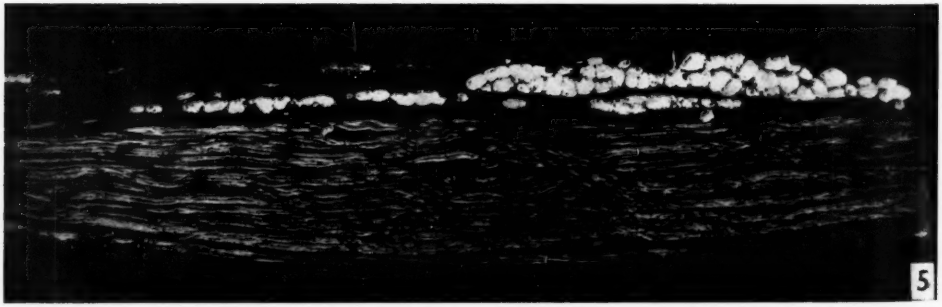
Weiss, P. & Taylor, A. C. (1944). Further experimental evidence against 'neurotropism' in nerve regeneration. *J. exp. Zool.* **95**, 233–256.

Wilkinson, H. J. (1929). The innervation of striated muscle. *Med. J. Aust.* **2**, 768–793.









AITKEN—Peripheral connexions on regenerating nerve fibres

EXPLANATION OF PLATE

All figures are of specimens fixed in Flemming's solution, Weigert-stained and photographed directly on to bromide paper.

Fig. 1. Regenerating nervus gastrocnemii medialis immediately proximal to the neuroma (185*e*). (×150.)

Fig. 2. Regenerating nervus gastrocnemii medialis 2 weeks after removal of the neuroma (185*b*). (×150.)

Fig. 3. Regenerating nervus gastrocnemii medialis which was implanted into a normally innervated muscle (642*c*). (×150.)

Fig. 4. Regenerating nervus gastrocnemii medialis which was implanted into a paralysed (denervated) muscle (642*a*). (×150.)

Fig. 5. Longitudinal section of union between nervus gastrocnemii medialis and n. suralis 95*d*). (×55.)

REVIEWS

Gynaecological and Obstetrical Anatomy. By C. F. V. Smout, M.D., M.R.C.S. and F. Jacoby, M.D., Ph.D. 2nd ed. (Demy 8vo. Pp. xi+248. With 185 illustrations. 40*s*.) London: Arnold.

The present book is in effect a second edition of *The Anatomy of the Female Pelvis* first published in 1943.

The book is divided into nineteen chapters and gives an account of the anatomy of the pelvis, the development and histology of the female genital tract, endocrine function of the ovarian hormones, the placenta, and the anatomy of the foetus in relation to childbirth.

The chapters describing the anatomy of the pelvis are concise and accurate, but not quite as comprehensive as one would have wished to have in such a volume. For example, no reference is made to the masterly contribution by Dickinson on 'Human Sex Anatomy'. Some of the rather elaborate diagrams are too schematic. For example, Fig. 96 shows the vagina as a straight tubular structure, whereas in fact it should be shown as curved in the sagittal plane, as in Fig. 97.

The chapters dealing with the histology of the ovary and with ovarian function and its control give a valuable account of the advances that have been made in these fields during the past decade. As this book is concerned mainly with human anatomy and histology it would have been more appropriate to have had more illustrations of the human ovary than of the cat.

The book has been accepted as a standard text-book and it is likely to have a long run with successions of editions. For this reason the reviewer has suggested the above comments for consideration by the authors. The book will be of value to those who are desirous of having a concise account of the anatomy and physiology of the female pelvis in one volume.

W. J. HAMILTON

Chronic Structural Low-backache due to Low-back Structural Derangement. By R. A. Roberts, B.Sc., M.B., Ch.B., D.M.R.E. (Pp. 105 (exclusive of 45 plates with 137 illustrations), 1947, 45*s*.) London: H. K. Lewis and Co., Ltd.

This is a valuable book for all concerned with differential diagnosis in patients with low-backache. Sixty-four case-histories are presented, the great majority of which show defects in the vertebral neural-arch, duly illustrated radiologically. The term pars interarticularis or isthmus of the neural-arch is defined as the narrowest and most delicate part of the neural-arch in the lumbar spine situated between the superior and inferior articular processes. The author considers this isthmus the vital pivotal centre in the architecture of the vertebrae for the interaction of the powerful muscles and ligaments of this region, because first, in the antero-posterior plane it is the pivotal centre between the pedicle with the body, and the lamina with the spinous process; secondly, in the vertical plane it lies between the superior and inferior articular processes which are most concerned with guiding the vertebral movements, and thirdly, in the lateral horizontal plane it is at the base of the transverse process.

The literature of separate neural-arch is discussed. Although absent in the foetus, its incidence, generally accepted at 5%, has been found up to 10% and even 25%. There must be many individuals with separate neural-arches in the last lumbar vertebrae who have never suffered low-backache. Dr Roberts submits the view that these defects are primarily the result of overstrain de-ossification, and are indeed akin to 'march' fracture or 'stress' fracture. In view of the fact that at least one highly competent radiologist has previously reiterated that such defects could not be demonstrated by X-ray, Dr Roberts is

to be congratulated upon producing the radiological evidence of these bilateral and unilateral defects of the pars interarticularis. He insists that oblique lateral views at about 30° from the antero-posterior position are essential for their demonstration. These deformities in themselves are not the cause of all the symptoms presented by the patients, but they indicate a structural defect, a flail joint, which must be counteracted by the soft tissues which are more susceptible to overstrain. He concludes that trauma is only an incidental aggravating factor in these cases; two-thirds of the cases did not reach their early twenties without suffering pain in the back. The oedema arising from overstrained soft tissues with consequent nerve pressure, is held responsible for the pain. In discussing neuro-vascular and visceral symptoms presented by patients, the author states that 'skeletal overstrain can affect the nearby sympathetic nerves causing anterior and visceral neuro-vascular disturbance'.

The histories of the patients have been carefully elicited, and the tragedies recounted of men of athletic activity and patriotic spirit, who have suffered the stigma of malingerer, neurotic and even mental defective, are well calculated to cause the clinician to ponder gravely and pursue his case-history and technical investigation exhaustively, before committing himself to diagnosis in these difficult cases.

We may sympathize with the author's question 'Since most of the present authoritative writings by gynaecologists, urologists, abdominal surgeons, "internists" and psychiatrists pay little attention either to the pain of spinal origin which may simulate visceral pain, or to the neuro-vascular symptoms which can arise through sympathetic irritation by skeletal lesions, is there not a need for a radical reassessment of our differential diagnoses?'

Dr Roberts agrees with many of his predecessors that these troubles are ultimately due to man's adoption of the erect posture. Surely this theme has been long overdone. The more we study anatomy the more we marvel at the excellence of Nature's handiwork, but certainly it is a surprise that more slips do not occur during the embryonic adventures of any animal in attaining the standard pattern. It may be that many anatomists and clinicians will consider that the author sometimes overstretches his thesis, but that is to be expected in the introduction of a doctrine.

Examination of the radiographs clinches the argument in favour of those who maintain that their interpretation is strictly a matter for the expert radiologist. Indeed, as a side issue to the teacher of anatomy, it is a debatable point whether the substitution to his students of the shadow for the substance, has not already gone beyond reasonable limits.

A minor point of criticism—possibly due to the case-histories having been culled from field records—is that the average reader is probably still pedantic enough to object to such abbreviations as 'Pain wakes him at night, gets up and walks about for a while'.

The format of the publication is excellent.

R. D. LOCKHART

Cardiovascular Studies. By KENNETH J. FRANKLIN, D.M., F.R.C.P. (1948. Pp. xvi +306. With frontispiece and 102 figures.) Oxford. Blackwell Scientific Publications.

This is an interesting work. It is written with the scholarship and erudition which mark all Prof. Franklin's writings, though we think he is at his best in Part I, a historical 'note' running to 47 pages, on the Eustachian valve and the intervenous tubercle of Lower. Apart from the smoothly flowing and felicitously worded narrative, the author brings to this historical study an enthusiasm which is contagious, and which whets our appetite for the rest of the book. Part II has not quite the unity and cohesion of Part I, for it consists of miscellaneous elements, including a brief discussion of terminology, a description of techniques, an account of how the essential ideas underlying the book developed, references to the literature, and observations on the human heart. On the question of terminology, we must confess to some unhappiness over the author's substitution of 'crista interveniens' for the intervenous tubercle.

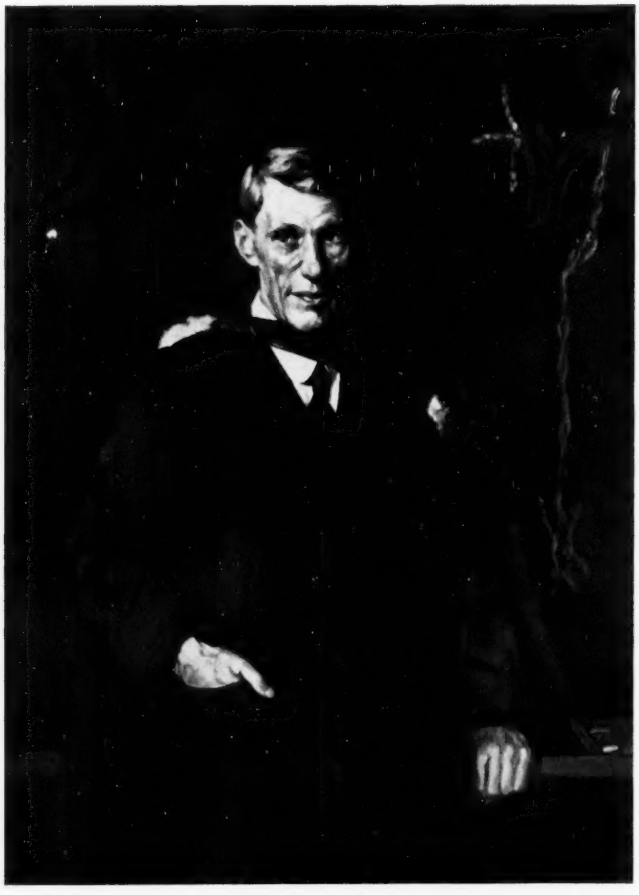
Part III constitutes the main bulk of the book, and consists of references to the literature and detailed observations on a large number of mammalian hearts. This part cannot be read in the ordinary sense of the word, but it is an exceedingly valuable compilation of factual material for reference purposes. Apart from the great mass of descriptive matter in Part III, matter which will assuredly be of permanent value irrespective of the author's interpretation of it, his main concern is with the significance and formation of the Eustachian valve. For this purpose he introduces two novel criteria for classifying hearts, namely the Heart Type, and the Heart Index.

The Heart Type is determined by the direction of blood flow in the superior vena cava, which on entering the heart may impinge: (*a*) on the caudal floor of the right atrium immediately to the atrial side of the Eustachian valve, the medial cornu of which is attached either to the surface of the fossa ovalis dorsal to the sternal pillar of its limbus [type I], or to the sternal pillar [type II]; (*b*) on the caudal floor of the right atrium between the Eustachian valve and the right atrio-ventricular orifice [type III]; (*c*) directly into the right atrio-ventricular orifice [type IV]. The Heart Index is the

$$\frac{\text{maximum height of Eustachian valve}}{\text{heart length}} \times 100,$$

and indicates the degree of development of the Eustachian valve; it should perhaps be more aptly designated the 'Eustachian valve index'. The main thesis of the book is that there is a correlation between Heart Type and Heart Index, and that furthermore the formation of the valve is stimulated haemodynamically, its height depending upon the angle of impingement on the atrial floor of the blood of the superior vena cava. This is an interesting concept, though one which it is difficult to submit to experimental verification. However, we agree with the author that the validity or otherwise of this concept will not 'reduce the general value of the book as a contribution to the comparative anatomy of the mammalian heart, and as an introduction to the literature on that and on allied subjects'.

Any review of this book would be incomplete if it failed to draw attention to the fact that the author, a Professor of Physiology, has contributed a study which at first sight appears to follow the tradition of classical morphology. Throughout the book, however, he is writing against the background of the experimental studies so brilliantly conducted by himself and others, and is thus enabled to arrive at a firmly grounded functional evaluation of his observations. Prof. Franklin's work represents an admirable illustration of harmonious combination in the study of structure and function. There are anatomists to whom function merely spells a series of airy generalizations and speculations based on scanty morphological observations. Not so the author of this book. By his assiduous devotion to morphology on the one hand, and the avidity with which he employs the most effective experimental techniques available on the other, Prof. Franklin has shown himself to be a worthy modern exponent of the true Hunterian tradition. J. M. YOFFEY



JOHN KAY JAMIESON

From the portrait in the Leeds University School of Medicine, by Mr Leo Whelan, Dublin, 1937

IN MEMORIAM

JOHN KAY JAMIESON, M.B., C.M. (Edin.); LL.D. (Leeds); M.A. (Dublin); M.CH. (Dublin), 1873–1948

John Kay Jamieson, Professor of Anatomy in the University of Leeds for twenty-six years and in the University of Dublin for another eleven, died at Dublin on 20 August 1948, aged 75. By his death the Anatomical Society loses one of its oldest members, as he was elected at the famous Summer Meeting of 1897, in Trinity College, Dublin.

Born in Shetland in 1873, he was the fourth son of Robert Jamieson of Sandness, other members of whose family also distinguished themselves academically. He attended the Sandness Madras School which his father had established before the Scottish Education Act of 1872 with the assistance, it is understood, of the trustees of the Rev. Dr Andrew Bell (1753–1832), himself the founder of the Madras College at St Andrews. Jamieson's eldest brother, Francis, after a period as Lecturer in Humanity in the University of Edinburgh, became H.M. Chief Inspector of Schools for Scotland; his two younger brothers entered the medical profession—Dr E. B. Jamieson, until lately Senior Lecturer in Anatomy, University of Edinburgh, and Dr J. P. S. Jamieson of Nelson, New Zealand.

In 1894, after graduating M.B., C.M. at the University of Edinburgh, Jamieson took up his first post as Demonstrator of Anatomy with Macdonald Brown, Lecturer in the Extra-mural School of Medicine, at Surgeons' Hall. Next year he was appointed Demonstrator under Wardrop Griffith at Leeds, and so began a period of 41 years' service to the School of Medicine there. In 1910, when Griffith was translated to the Chair of Medicine, Jamieson was the natural choice to succeed him as the first whole-time Professor of Anatomy in the University of Leeds.

From 1910 to 1914 he served as Sub-Dean of the Faculty of Medicine and was Resident Warden of Lyddon Hall, the hostel for men students. But it was during the 1914–18 War that his talents as administrator led to serious demands on his time and energy which were thus heavily mortgaged to the conduct of affairs throughout the rest of his period in Leeds. From 1914 to 1918 he acted as Dean of the Faculty of Medicine and was Registrar, later Commanding Officer with the rank of Lieut.-Colonel, of the East Leeds War Hospital. In 1918 he was formally appointed Dean of the Faculty, an office which he retained until he left Leeds in 1936. During these eighteen years he was continuously a member of the University Council; he was Pro-Vice-Chancellor 1923–25 and in 1923–24 Acting Vice-Chancellor of the University. From 1928 to 1936 he also represented the University of Leeds on the General Medical Council; and it is not surprising that he came to be regarded as the dominant personality in the Leeds School of Medicine.

Jamieson's outstanding services to the University of Leeds and to its School of Medicine in particular have indeed been handsomely acknowledged by one of his colleagues (*Lancet*, 18 September 1948, p. 479, and *Leeds University Review*, 1948). Here it is sufficient to record that he was the chief architect of the great development of the Leeds School of Medicine during the inter-war years—the establishment of

a new School of Dentistry in close relation to the General Infirmary (1928), the extension of the Department of Physiology (1930), with subsequent provision of Departments and Chairs of Biochemistry and Pharmacology, and the building of the Algernon Firth Institute of Pathology (1933). On the occasion of his departure from Leeds for Dublin in 1936, the University of Leeds also acknowledged its debt to Jamieson by conferring on him the degree of LL.D. and by the presentation of a characteristic portrait that now hangs in the Library of the School of Medicine.

For in 1936, to the surprise and regret of all his colleagues, Jamieson decided to accept an invitation from the University of Dublin to succeed A. Francis Dixon in the combined chairs of Anatomy and Chirurgery and of Human Anatomy and Embryology. A transfer from one University to another after 41 years' service is unusual; but Jamieson was then approaching the normal age of retirement at Leeds, and Trinity College offered him an additional five years' service to Anatomy unburdened by excessive administrative demands. In the event, owing to the outbreak of war and other factors, he remained in office at Trinity College for eleven years until his final retirement in December 1947. The University of Dublin admitted him to the degrees of M.A. in 1938 and of M.Ch. in 1947, the latter degree an unusual distinction indicating the high value set on his work by his surgical colleagues.

Jamieson was no stranger to Trinity College and Dublin; he was welcomed there as a friend, and he quickly made an honoured place for himself in academic and medical circles and in the affection of his students and colleagues (*Lancet*, 4 September 1948, p. 397). He was at once elected a Fellow of the Royal Academy of Medicine in Ireland, and he served later as President of the Section of Anatomy and Physiology. He took a great interest in the well-being of that Section—his usual pithy comments became a feature of its discussions—and he contributed two papers to its *Proceedings*: 'The distribution of lymphatics' (1936–37) and 'The teaching of Anatomy' (1941–42). In relation to his older Chair of Anatomy and Chirurgery, which originally carried beds in Sir Patrick Dun's Hospital in Dublin, he was appointed Consulting Anatomist to that hospital; and he was elected also a Member of the Royal Irish Academy (1937).

As a teacher of Anatomy Jamieson's reputation was early established, and there is abundant testimony that it stands very high with his former students at both Leeds and Dublin. He was a regular attender at the Leeds and West Riding Medico-Chirurgical Society, of which he was made an honorary life-member—a rare distinction, and his teaching was strongly influenced by practical clinical considerations in the Leeds tradition. His lectures on Anatomy in relation to function and to practice, delivered in a very characteristic style and abundantly illustrated by simple diagrams, held the attention of his students and made them think for themselves. He excelled in the intimate teaching of the dissecting room, and he took great delight in the demonstrations that he continued to give there during the whole period of his professorships.

As an examiner Jamieson had a very wide experience and was adept in assessing a candidate's knowledge in a short space of time. He had examined in most of the British Universities; and during the 1914–18 War, and for some time after, he served continuously as External Examiner at Trinity College so that, as has been said, he was no stranger when he arrived in Dublin in 1936. At one period during the Second World War, owing to difficulties of travel, he

enjoyed, and confessed that he enjoyed, the unique distinction of examining in *all* the Schools in Ireland.

Jamieson's well-known contributions to the Surgical Anatomy of the Lymphatic System—made principally before he was immersed in administrative duties—have stood the test of time. The reputation of Leeds in Surgery and the fact that a number of rising young surgeons, including Berkeley Moynihan, were Honorary Demonstrators of Anatomy and among his first friends in Leeds, directed his interest to investigations that would assist in surgical advance. With the late Mr J. F. Dobson he was early engaged in the time-consuming labour of investigating the lymph-drainage of various regions and organs by means of Gerota's method, of which they published a brief account: 'On the injection of lymphatics by Prussian blue' (*J. Anat.* 1911, **45**, 7). The main fruit of these labours was a series of papers that began to appear in 1907, first as the Arris and Gale Lectures on 'The lymphatic system of the stomach' (*Lancet*, 1907, **1**, 1061) and 'The lymphatic system of the caecum and appendix' (*ibid.* 1907, **1**, 1137), followed by 'Excision of the caecum and ascending colon with the corresponding lymphatic area' (*ibid.* 1908, **1**, 149), 'The lymphatics of the colon' (*Proc. Roy. Soc. Med.* 1909, **2**, 149), in which their well-known grouping of the colic lymph-glands was described, and 'The lymphatics of the testicle' (*Lancet*, 1910, **1**, 493). At the Summer Meeting of the Anatomical Society in July 1907 at Edinburgh, they had given a 'Demonstration of lymphatic vessels and glands injected according to the method of Gerota' (*J. Anat.* 1909, **43**, *Proc.* p. ii) and, after the interruption of the 1914–18 War, the last paper in this notable series appeared: 'The lymphatics of the tongue: with particular reference to the removal of lymphatic glands in cancer of the tongue' (*Brit. J. Surg.* 1920, **8**, 80). In that paper they named the jugulo-omohyoid gland as a companion to the jugulo-digastric gland of Leaf—limiting between them the deep cervical glands that receive lymph directly from the tongue—and they said the last word about the lymph-drainage of that organ while operative excision was still the treatment of first choice.

Although radio-therapy may tend to replace operative surgery in the treatment of malignant disease, these studies of Jamieson and Dobson in the Surgical Anatomy of lymphatic drainage retain their basic value; and the usefulness of their illustrations to students is testified by their inclusion in our standard text-books.

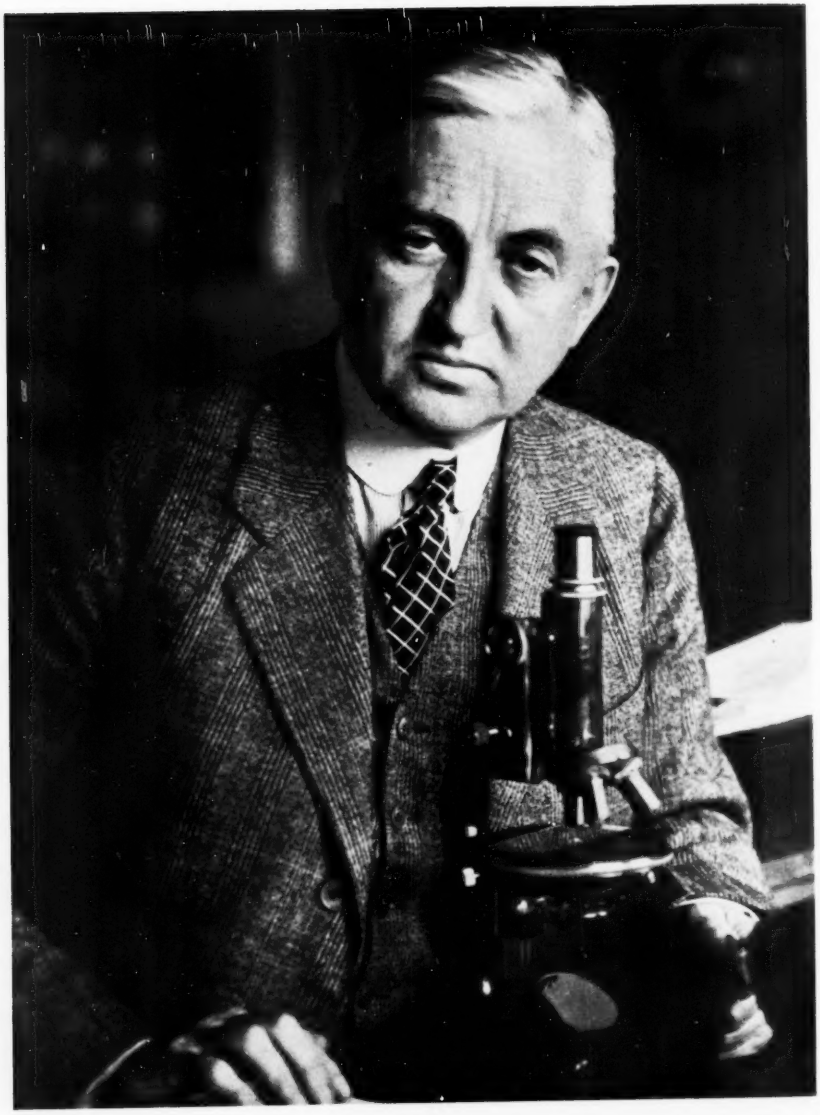
Jamieson, like many other anatomists, was an open-air man in such spare time as he had. He was an ardent golfer—and we use the adjective advisedly. His walk was always characteristically rapid and, so far as the field in front of him would allow, he raced round a golf course as if there were no time to lose, his partners and opponents sometimes hard put to it to keep up with him. He was a regular player at the Alwoodley Club in Leeds and he joined the Island Golf Club at Dublin. He took part in all the Staff Competitions at Trinity College and won the Staff Golf Cup twice. It was one of his chief regrets, though he made light of it, that increasing disabilities in later years prevented him enjoying the strenuous exercise on the golf course to which he had been accustomed.

Of Jamieson the man, a personal appreciation by one of us has already appeared (*Lancet*, 4 September 1948, p. 398), and a striking testimony to his popularity among his students and the esteem in which they held him has been contributed by one of themselves (*Brit. med. J.* 11 September 1948, p. 535). With his Norse origin, he was

a man of arresting personality that instantly impressed all with whom he came in contact. On further acquaintance, as his rugged appearance and his unconventional outlook and manners became familiar, few could resist the kindly disposition and the essential friendliness that they imperfectly concealed. Well read in classical literature, he was steeped in his native lore, and he had a fund of curious information on surprising subjects. His conversation was not only always stimulating but also on occasion surprising too. He had the most penetrating judgement of men and their motives; and his advice, sought by many in private as well as in public affairs, was invariably shrewd and solid. Inevitably he had a large circle of friends, and he was an indefatigable and lively correspondent, increasingly so after his transfer to Dublin: his letters were the kind that one keeps—informative, amusing, gossipy, often cryptic and not infrequently pungent in their comments on local and other affairs. It may confidently be said that John Kay Jamieson will always be gratefully remembered by his pupils, his colleagues and his friends as a notable and remarkable figure, whom to have known has indeed been a liberal education.

Professor Jamieson married Elisabeth, daughter of Dr R. P. Goodworth. Her kindly welcome is a happy memory of all former members of her husband's Staff at Leeds. She died there in 1936—her loss may well have affected his decision to move to Dublin—and they leave a daughter and a son, Dr John Jamieson, to whom we extend our sincere sympathy.

J. C. B.
R. G. I.



GEORGE LINIUS STREETER

IN MEMORIAM

GEORGE LINIUS STREETER, A.B., A.M., M.D., D.Sc., Sc.D. (Dublin), LL.D. (Michigan), F.R.S. Edinb.

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Research Assistant, Carnegie Institution of Washington
Director of the Carnegie Laboratory of Embryology, Baltimore
Member of many Learned Societies
Honorary member of the Anatomical Society of Great Britain and Ireland

The sudden death, at the age of 75, of Dr George Linius Streeter has removed from the contemporary field of human embryologists its greatest figure. Dr Streeter was trained in the great tradition of descriptive embryology and neurology and his earlier work, summarized in Keibel and Mall's Manual, was concerned largely with the development of the human peripheral and central nervous systems. After 1914, however, when he left his position as head of the Anatomy Department at the University of Michigan to go to a full-time research post at the newly founded Carnegie Laboratory of Embryology in Baltimore, his embryological interests became much broader, or, at least, found an opportunity to reveal themselves more fully. Following the tragic death in 1917 of Prof. Mall, Dr Streeter became Director of the Laboratory which had already a great reputation, and which will always be associated with the name of its great founder Dr Mall. Under the directorship of Dr Streeter the laboratory grew from the unachieved brilliance of Mall's concept to its present position as the most important laboratory of embryology in the world.

This fulfilment of Mall's dream was due to Streeter's vision, energy and personality. He saw that such a laboratory must be more than a repository of human embryos. To fulfil the aims of the founder the experimental and comparative methods must be added to the descriptive approach, and the basis of embryological study—sex and reproductive physiology—had to be explored. Finally, in the background of all the work there had to be a broad biological interest in embryos as living organisms with historical impulses (which, for Streeter, were genetical) and structural adaptations necessary for coping with, or exploiting, the environment.

To achieve these ends Streeter gathered round him a brilliant group of investigators, the Lewises, Corner, Wislocki, Heuser, Hartman, Metz, Gey, Burn, Flexner. These, with collaborators from the Johns Hopkins Department of Anatomy, Hines, Schultz, Straus, Gersh, and a host of visiting workers from all over the world, under Dr Streeter's guidance, engaged in problems the full extent of which can be studied in *The Annual Report of the Director of the Department of Embryology of the Carnegie Institution of Washington*, and in the *Contributions to Embryology* of which vols. VIII to XXIX appeared under his editorship and which 'with self-effacing devotion, with adherence to the highest standards of technical perfection, and with the capacity

to enlist skilled collaboration on the part of scientific contributors, illustrators, printers and engravers, and the Institution's Office of Publications, made a veritable treasury of embryological progress, famous among the world's archives of science' (to quote from the prefatory note to vol. xxx of the *Contributions*, dedicated to him by his colleagues and other workers).

While he was concerned with providing facilities for all these workers, with the editing of their publications, with his duties as administrator of a large laboratory and with expanding the research collection of human embryos, Dr Streeter found time to do a great deal of personal investigation, either alone or in collaboration. His descriptions of the development of the internal ear and the cranial sinuses are classical. His surveys of early human developmental stages and of the Miller ovum consitute the basis for all the beautiful Hertig and Rock material which has revolutionized our knowledge of the early development of man. His work with Dr Heuser on the early stages of the pig and, especially, of the *Rhesus* monkey are fundamental. His last publications, on human developmental horizons, are characterized not only by a meticulous accuracy, but by a breadth of biological vision which is a revelation of the way in which embryology should be studied and presented. To these achievements can be added many other significant studies, such as those on twinning, developmental abnormalities and metamerism of the central nervous system.

To conduct a department of this magnitude, to carry out personal research, to attract workers from all over the world, to welcome and inspire them, required a man with a high sense of duty, a love for his fellows, a spirit of enthusiasm, unselfishness, simplicity and friendliness, a readiness to see another's point of view, and a sense of humour. These were the characteristics of Dr Streeter.

The photograph was taken by Mr F. J. Pittock in Prof. J. P. Hill's laboratory.

J. D. B.

C. M. W.

Anatomical Society of Great Britain and Ireland

PROCEEDINGS

November 1947
February 1948
April 1948
June 1948

RECORDED BY
W. F. HARPER

PROCEEDINGS OF THE ANATOMICAL SOCIETY

NOVEMBER 1947

The Annual General Meeting of the Society, for the Session 1947–48, was held on Friday, 28 November 1947, in the Anatomical Department, University College, Gower Street, London, the President (Professor A. B. APPLETON) in the Chair.

Professor J. Z. YOUNG presented a communication on 'Narrowing of nerve fibres at the nodes of Ranvier'.

Measurements of the thickness of the axon and myelin in serial sections through nodes of Ranvier of rabbit's nerves show that the axon becomes reduced, over a length of about 25 μ, to as little as a quarter of the diameter it shows along the internode. This constriction is produced by an increase on each side of the node in the thickness of the myelin, which may reach 7·5 μ compared with 2·5 μ over the rest of the fibre. The axon is thus reduced at this point to a narrow channel, about 5 μ in diameter in the case of a large fibre, surrounded by a myelin sheath of 7·5 μ thick. The axon also remains narrow in the node proper, the region where it is not surrounded by any myelin, the length of this being about 5 μ in the case of the largest fibres.

The great narrowing of the fibre at the node, together with the increase here of the myelin, may be responsible for some of the membrane-like properties which have been recorded as observable electrically at the nodes. No visible transverse membrane has been seen crossing the axon itself at the node, but the axoplasm may show slight differences of staining where it becomes narrow. The inturning of the neurilemma to make the cementing disc can also give appearances difficult to distinguish from those of a transverse membrane across the axon.

Professor LE GROS CLARK inquired if the constrictions observed in the preparations might not be artefacts, and Professor YOUNG replied that they did not occur elsewhere on the nerve fibres, and other workers had recorded similar appearances.

Professor MACCONAILL asked if narrowing of nerve fibres had been noted in other mammals, and was told that they had been observed but that the relative measurements of nodal and internodal regions differed in the various orders of Mammalia.

Dr WEDDELL inquired if there was any significant variation in the degree of narrowing of the fibres from one rabbit to another, and Professor YOUNG replied that he had not gone into this matter fully but that in the nerves of frogs he had observed some variation.

Dr J. JOSEPH read a paper on 'Quantitative nuclear changes in the peripheral stump of the greater splanchnic nerve of the rabbit'.

The greater splanchnic nerve of the rabbit is composed almost entirely of small myelinated fibres. In normal nerves the number of nuclei in right and left nerves and at two levels in the same nerve is not significantly different. There is, however, a significant difference between individual rabbits. After 21 days' degeneration, the peripheral stump shows an increase in the number of nuclei of the order of only 34%, as compared with 800% in a large myelinated nerve, and with 0% in a non-myelinated nerve. These differences may be explained by the quantity of degenerating myelin and/or the size of the fibres. Previous investigators attempted to relate the proliferation of the cells in the peripheral stump to regeneration, the proliferation being necessary either for a pathway for the growing axon or to produce myelin for the maturation of the fibres. At 21 days it is impossible to determine to which type of cell the increase in number is due.

Professor YOFFEY inquired if the author had made any specific tests for macrophages following degeneration. Dr JOSEPH replied that while it was impossible to distinguish between the different types of cell in a transverse section of the greater splanchnic nerve

at the end of 21 days' degeneration, two types of cell could be recognized after 1 week of degeneration. Comparison of the two might determine to what extent different cells had increased. He added that other investigators have stated that there is no increase in the number of macrophages in relation to degenerating small fibres.

Dr L. Chacko read a paper on 'The laminar pattern of the lateral geniculate body in Man and the lower primates'.

Reconstruction models of the geniculate body in Man, *Macaca*, *Lemur*, and *Microcebus* have been prepared. They show that in the primates the geniculate body varies from the completely 'inverted' type in which the small-celled laminae are contained within the concavity of the large-celled laminae, to the completely 'everted' type in which the large-celled laminae are contained within the concavity of the small-celled laminae. The laminar pattern in Man has been studied in relation to the projection of the central and peripheral fields of the retina on the geniculate body, and also with special reference to the distribution in the retinal field of colour sensitivity. Finally, the total number of cells in each set of laminae of the human geniculate body has been computed from serial sections, from which it appears that the ratio of crossed to uncrossed fibres in the optic tract is approximately 60 to 40.

Professor Le Gros Clark, commenting on the functional significance of the laminar pattern, emphasized that a recording of electrical responses from localized areas of the geniculate body during the stimulation of the retina with monochromatic light might help in the elucidation of the problem. Dr Chacko remarked that a record of the areas of degeneration of the geniculate nucleus after prolonged exposure of the retina to monochromatic light, and a detailed analysis of the nucleus in cases of colour scotoma might also help.

Dr W. H. Feindel read a paper on 'The pattern of innervation in the muscle-spindle of the rabbit'.

Using a modification of the intravital methylene-blue technique it has been possible to demonstrate the entire pattern of the intramuscular nerve supply of a lumbrical muscle in the rabbit. In such a preparation it is possible to study the relationship of the various nerve fibres and endings concerned with the innervation of the muscle spindles, for the muscle is small enough to allow complete examination without sectioning.

It has been found that the muscle-spindles are supplied by one large myelinated nerve fibre and by at least four smaller fibres, and the distribution of these in relation to the intrafusal muscle fibres has been defined. The large myelinated fibre, shown by Sherrington (*J. Physiol.* 1894, **17**, 211–250) to be afferent in nature and by Lloyd (*J. Neurophysiol.* 1943, **6**, 317–326) to subserve the stretch reflex, has been traced to a single fibre directly from one of the muscle-spindles to the main nerve bundle. The terminations within the muscle-spindle of the smaller nerve fibres show considerable variation in morphology and in many cases are supplied by at least two axons.

Dr Weddell congratulated the author on his development of the new intravenous methylene-blue method for studying the nervous system.

Dr Barker asked if the diameters of the large afferent nerve fibres both at the spindle and in the main nerve to the muscle had been measured. Dr Feindel replied that while he had not yet made careful measurements there seemed to be no great discrepancy in diameter as one traced them from the spindle to the main muscle nerve. They remained throughout about twice the diameter of the next largest fibres and were, therefore, the only possible fibres which could act as the rapidly conducting afferent arc of the stretch reflex. He also pointed out that the arrangement of a large afferent fibre and motor fibre running together without branching from the end-organ to the main muscle nerve was found as well in relation to the tendon-organ, and that either or both of the organized endings might be concerned in the stretch reflex.

Dr C. H. A. WEDELES (introduced by Professor J. Z. YOUNG) read a paper on 'The effect of increasing the functional load of a muscle on the composition of its motor nerve'.

This experiment is part of an investigation designed to ascertain the influence of peripheral connexions on the structure of motor fibres. It is now known that the peripheral, as well as the central connexion, is an essential contributor to the restoration of injured nerves. Normalization of fibre number and diameter proceeds imperfectly with severed or non-functioning distal connexions.

The medial belly of the gastrocnemius muscle of the rabbit on one side was made to perform extra work by denervating the lateral belly, plantaris and soleus, the other side serving as control. After 100 days the overloaded normal nerve showed an increase both of the total number of its constituent fibres and in its total fibre area. There is a shift of fibres into larger size groups, and previously 'non-medullated' fibres acquire myelin sheaths.

The effect of overloading a nerve during regeneration was also studied by crushing the nerve to the medial head of gastrocnemius on both sides and denervating the other muscles as before on one side only. After 100 days of regeneration the overloaded nerves showed a considerably greater number of fibres, but these were not larger than normal.

Professor WEST inquired as to the source of the additional nerve fibres and was informed that the author was quite ignorant as to their origin or function. Dr WEDELES suggested, however, that either the so-called 'unmyelinated' fibres had acquired a thicker myelin sheath and so become visible by the Weigert-Pal technique, or that there had occurred a branching of existing fibres in response to muscle hyperplasia. There are no facts to prove either assumption and further work must be done to elucidate the problem.

Professor YOUNG remarked that in any case the increase in the number of new nerve fibres was not large.

Dr B. FRANKENHAEUSER read a paper entitled 'A method of determining the cutaneous distribution of a sensory nerve by means of action potential recording'.

The cutaneous distribution of the saphenus minor (sural) nerve in the rabbit has been determined by recording the action potentials produced in the nerve following stimulation of the hairs. The hairs were stimulated with a no. 3 nylon suture mounted in a metal holder. The action potentials were led off from the exposed nerve by two silver-silver chloride electrodes feeding into separate amplifiers. Stimuli outside the area of distribution of the nerve gave rise to no action potentials, but when stimuli were applied to the autonomous zone several spikes were recorded. Stimuli applied to the intermediate zone produced a few spikes only. It appears that when the nerve has suffered no damage every spike visible in the record from one electrode is also visible in the record from the other. The cutaneous distribution of the saphenus minor nerve so outlined is in general agreement with that previously found by Weddell, Gutmann & Gutmann (1941).

It is submitted that this method of outlining sensory areas in the skin, in which the physiological integrity of the nerve and its cutaneous end-organs is preserved, is more valid than the older destructive procedures, and therefore has a place in the anatomical armamentarium.

Dr WEDDELL and Professor J. Z. YOUNG commented on the clinical importance of this new method for demarcating sensory areas in the skin.

Professor M. A. MACCONAILL read a paper on 'The myelothecal apparatus of the axis cylinder'. He described a method of staining the C.N.S. with lead as follows:

The myelothecal apparatus is stained with complete regularity by the method described below. It consists of a thin membrane, seemingly continuous, forming an immediate sheath to the medullated part of the axis cylinder, i.e. it is internal to the myelin. This is the primary part. The secondary part consists of a well-marked spongework, extending across the myelin space, and forming a reticulum on the surface of the myelin sheath proper. It appears to be elastic. This part is specially developed to form the infundibula at the incisures of Schmidt-Lantermann. The primary part is present within and without the brain and

cord; but the secondary part is found in peripheral nerves only. The development of the primary part appears to proceed *pari passu* with medullation.

Paraffin sections are brought through the alcohols to a saturated solution of picric acid (2 min.). They are then immersed in acid fuchsin (1 %, 10 min.) and then in lead haematoxylin (made by mixing equal parts of aqueous solutions of haematoxylin (2 %) and lead nitrate (5 %); tap water may be used; the mixture is filtered and allowed to ripen for 1 or 2 days); (4 min.). Lastly, they are placed in saturated ammonium molybdate solution for 1 min. The sections are, of course, washed well (tap water) between each of the foregoing stages. The times given are for 6 μ sections; they should be halved for 12 μ sections, except for the ammonium molybdate bath. Results: nerve cells and dendrites, dark brown; nucleoli of *nerve cells*, red; axis cylinders, dark blue to black; myelothecal apparatus, bright red; collagen, dull pink; red blood corpuscles, rose-red.

Dr R. G. Harrison remarked that he had hitherto agreed with Nageotte that networks of the character demonstrated were artefacts, but now thought otherwise as a result of the series of differences between the various types of nerve fibres shown by Professor MacConaill.

Professor H. Barcroft and Dr C. T. C. Hamilton (introduced by Professor J. Z. Young) read a paper entitled 'Results of sympathectomy in the upper limb'.

(1) Examination of seventeen upper limbs 1–6 months after sympathectomy (preganglionic section) showed: (*a*) Marked clinical improvement. No recurrence of the attacks in fourteen out of sixteen hands operated on for Raynaud's disease. In two hands the attacks were less frequent and less severe. (*b*) That vasometer and sudometer reflexes were absent. (*c*) That therefore preganglionic section equals complete sympathectomy of the limb. Th. 1, which is not cut, does not convey sympathetic fibres to the hand.

(2) Examination of a further thirty-six upper limbs 1–6 years after preganglionic section showed: (*a*) Marked clinical improvement. No recurrence of vasospastic attacks in eighteen out of thirty-six limbs operated on for Raynaud's disease. In fourteen hands the attacks were much less crippling. In only four had no benefit been obtained. (*b*) That vasomotor and sudomotor reflexes were absent in some limbs, but present in many. (*c*) That nervous connexion between brain and the vessels and sweat glands of the hands is often re-established. (*d*) That the persistence of the excellent clinical results in spite of the re-establishment of a vasomotor path probably signifies that the new path is functionally inferior to the original one. (*e*) That partial regeneration is the most likely explanation of the new nerve path.

Mr K. C. Richardson read a paper on 'The problem of contractile tissues in the mammary gland'.

Four components of the mammary stroma may be involved in varying degrees in the mechanical transfer of milk from the alveoli to the ducts and cisterns, but recent physiological investigations suggest that the myoepithelial cells, associated with the alveolar epithelium, are the main contractile component, and that smooth muscle bundles, elastic fibres and blood vessels are of minor importance. The position, number and morphology of the myoepithelial cells in the goat mamma have been examined in preparation for further experiments on the structural changes in the udder associated with the phenomenon of 'let-down'.

Dr Glees inquired whether there was not some possibility of confusion between reticular fibres and myoepithelial cells. Some of the silver preparations shown reminded him of stained argentophile fibres and he wondered how one was able to discriminate between these and contractile elements. Mr Richardson replied that the structures outlined by silver impregnation in the mammary gland were undoubtedly cells, similar in size, distribution and location to the myoepithelium revealed by routine staining. Moreover, the silver technique could be controlled by adjusting the pH so as to stain myoepithelium, smooth muscle and connective tissue fibres separately without any possible confusion.

Dr D. H. L. Evans read a paper on 'Some problems in enteric innervation'.

The enteric plexuses of the cat, rabbit and frog stained with Bielschowsky-Gros, Cajal and methylene-blue methods show that the interstitial cells of Cajal occur not only in relation to the enteric plexuses but also throughout the circular muscle coat. They form the web-like structure of the plexuses in which the nerve fibres run, and thus resemble Schwann cells. Beyond the plexuses methylene-blue preparations show nerve fibres running in the processes of interstitial cells in their course towards the muscle elements. Cajal preparations of the circular muscle show columns of nerves running parallel to the muscle fibres and distributing branches which run close to the muscle cells. No undoubted endings were found, and it seems possible that a functional synapse is established over an extensive length of nerve fibre as it runs adjacent to several muscle cells. No peripheral network was seen, such as would be expected on the theory that post-ganglionic fibres anastomose to form a syncytium at their terminations.

Dr Glees commented on Stoehr's conception of the terminal reticulum. They were not artefacts as had been maintained by Nonidez but represented terminal nervous arborizations. The Cajal preparations demonstrated by Dr Evans did not show a complete staining of all nerve fibres. Cajal's technique was unsuitable for the peripheral nervous system. The only hope of getting some agreement in this difficult field of research would be for the two opposing schools to meet and discuss their conceptions.

Professor Sosa said that in silver preparations, either with Cajal, Bielschowsky-Gros or Golgi methods, or in Ehrlich's methylene-blue preparations, he had never found the so-called 'terminal reticulum' as a real nerve structure. When it did appear under some technical conditions, it could be interpreted as the result of a partial impregnation of pre-collagen fibrils. On the other hand, he had always seen the terminal innervation of smooth muscle fibres, in the intestine and other organs, made only by contact of terminal knobs or masses on the surface of the muscle cells as stated by Cajal.

Dr Brandt stated that in a recent monograph Hillarp concluded that the 'terminal reticulum' of a nerve fibre does not exist. In Auerbach's plexus in the gut of *Myxine* after the application of silver nitrate (Schultz-Gros method) ganglionic cells and nerve endings similar to those Dr Evans had shown, can be demonstrated. But there is no peripheral network.

Dr E. C. Amoroso in a paper entitled 'The vascular and endometrial relations of the placenta of the sow' discussed the manner of union of the trophoblast and the mucosa.

Dr Brandt remarked that in a recent number of the *Revue Suisse de Zoologie* Töndury had described the placenta of the sow in detail, noting specially the close relationship of the blood capillaries to the epithelium.

Professor Nicol congratulated the author on the excellence of his histological preparations and photomicrographs.

Dr A. Lasnitzki (introduced by Professor S. Zuckerman) read a paper on 'Investigations on the conversion of lymph-nodes into haemolymph-nodes'.

While the occurrence of haemolymph-nodes in the normal rat is confined to the renal and splenic regions, it has been found that, under experimental conditions, lymph-nodes in various other regions of the body can be converted gradually into haemolymph-nodes of more or less pronounced character. Changes of this nature were brought about, first, by the influence of a prolonged treatment with the carcinogenic hydrocarbon 1:2:5:6-dibenzanthracene, administered subcutaneously in the form of an aqueous colloidal solution. The haemolymph-nodes thus produced corresponded in their gross anatomical appearance and histological structure to those already present in untreated rats. Similar, although less striking, results were obtained if rats were treated similarly with two other carcinogenic hydrocarbons, 3:4-benspyrene and methylcholanthrene. On the other hand, the two non-carcinogenic hydrocarbons anthracene and phenanthrene, administered in a comparable manner, failed to produce any appreciable haemolymphatic changes.

It has been observed, further, that abnormally situated haemolymph-nodes, with similar morphological properties, also occurred in rats bearing transmissible tumours implanted under the skin (Jensen sarcoma). This action was supposed to be due to a substance released by the growing tumour, and subsequent experiments, undertaken from this point of view, showed that significant haemolymphatic changes, although less frequent and pronounced, could be obtained after subcutaneous administration of the total lipoids extracted from dried tumour tissue. The total lipoids contained in samples of human cancerous tissue had similar effects.

Dr J. WHILLIS and Dr G. D. CHANNELL presented a paper on 'The action of the lumbrical and interosseous muscles in some of the movements of the digits'.

Though for the past one hundred years the accepted view has been that the lumbricals produce extension of the interphalangeal joints by a direct pull on the extensor expansion, this is not really the case.

There are two natural movements in which extension of the interphalangeal joints occurs, viz: (1) opening the hand when extension occurs also at the metacarpo-phalangeal joints; and (2) the movement used in threading a needle where extension of the interphalangeal joints is accompanied by flexion of the metacarpo-phalangeal joints.

In opening the hand the movement is produced by active contraction of the extensor digitorum communis accompanied by reciprocal relaxation of the flexor muscles.

In the 'needle-threading' movement the extensor communis and the flexor muscles both maintain a constant tone. Flexion of the metacarpo-phalangeal joint by the lumbrical and interosseous muscles produces automatic slackening in the flexor tendons and tightening of the extensor communis which is expended in extending the interphalangeal joints, the role of the long tendons being that of inextensible passive ligaments.

The lumbricals also produce radial deviation and rotation of the digits when they are opposed to the thumb.

Their role in relaxing the flexor tendons in unnatural movements with the first phalanx fixed was discussed.

The results have been checked by the production of acute paralysis by injection of the radial and ulnar nerves and by direct stimulation of the lumbricals.

Professor MACCONAILL expressed his satisfaction at the facts established; they were confirmatory of conclusions drawn from the theoretical mechanics of the joints.

Dr J. MCKENZIE read a paper on 'The parotid gland in relation to the facial nerve'.

McWhorter (*Anat. Rec.* 1917, **12**, 149–154), describing the parotid gland as a bilobed structure, was the first to state that the larger lobe, lying superficial to the facial nerve, and the smaller, deep to the nerve, were joined by an 'isthmus' consisting of gland parenchyma or ducts or both, which passed between the two main divisions of the nerve, the temporo-facial and cervico-facial. Recently Hamilton Bailey (*Brit. med. J.* 1947, **1**, 404–407), confirming these views, stated 'There can be no doubt that surgical enterprise in the parotid region has been stultified by the instruction that surgeons have received and are receiving from anatomists'.

The present series of dissections which, it is hoped, will refute these charges, shows that further communications occur between the two portions of the gland in addition to those previously described. Small ducts have been observed passing between the smaller branches of the facial nerve from lobules of the gland lying deep to the nerve, lobules which have been accepted as part of the deep 'lobe' of the gland by McWhorter, Roubiere & Cordier (1934), McCormack, Cauldwell & Anson (1945) and others.

The PRESIDENT said that he agreed with the conclusions drawn from the dissections and raised the question as to the relation of the parotid gland to the facial nerve in early foetal life. Dr MCKENZIE was unable to give any information on this point, as he had not studied this aspect of the problem.

Dr R. J. HARRISON read a paper on 'The vascular supply to the human testis'.

The blood supply of the testis as demonstrated by radiography and micro-arteriography was described. The common statement that the testicular artery passes into the substance of the testis at the mediastinum is found not to be true. The commonest arrangement is for the artery, on reaching the posterior border of the testis, to divide into two branches, both of which pass into the tunica albuginea to ramify in the tunica vasculosa over the lateral and medial surfaces respectively. The terminal branches are given off from these superficial branches at various points over the free surface of the testis and pass in towards the mediastinum, commonly bending back again to pass centrifugally before reaching it. The veins draining the testis are arranged as small, tortuous, superficial veins, deep veins, and large short-circuiting anastomotic channels.

Reference was made to the vascular pattern in the testis of other animals, particularly the wallaby, *Macropus rufogrisea fruticus*, in which a sort of 'rete mirabile' is formed in the spermatic cord by the testicular artery.

Professor CAVE inquired whether any indication of the distribution of the artery to the gubernaculum in the testis had been observed. Dr HARRISON replied that this had not been seen, but that the next obvious problem was the elucidation of the distribution of other arteries, e.g. the artery of the vas.

Dr R. BARER read a paper on 'Phase contrast microscopy in Anatomy'.

Some applications of phase contrast microscopy to anatomical problems are described. The method is invaluable for the examination of living material both as isolated cells or in the living intact organism. Transparency, rather than actual thickness of the specimen, is the limiting factor in its use. Contrary to what is often stated, the method may be extremely useful as an accessory to routine examination of fixed and stained sections. Elements which may have been poorly stained can often be rendered clearly visible. A detailed investigation of the effects of fixatives and routine histological procedures on living cells is now in progress.

Professor MACCONAILL remarked that the microphotographs of nerve cells and of the cerebellar cortex shown were remarkably like his own preparations obtained by the lead haematoxylin technique described earlier in the meeting.

Dr W. F. HARPER read a paper entitled 'Further observations on the blood vessels of the nasal mucous membrane in mammals'.

In a previous communication the author has reported the presence of arterio-venous anastomoses in the human nasal mucosa. Observations are now presented on the location, relative number and structure of these anastomotic cross-connexions between the arterioles and the venous blood spaces in the mucosa of the human inferior turbinate. Additional data concerning the structure of the walls of the venous blood spaces, the intra-osseous veins of the inferior turbinate and the so-called 'Drosselvenen' of Korner (*Z. mikr. Anat. Forsch.* 1936, **41**, 131) are given and discussed with reference to the erectile capacity of the mucosa.

The blood vascular patterns of the turbinate mucosa in the cat, rabbit and certain ungulates are described and compared with that of Man.

An interesting type of arterio-venous communication occurs in the turbinate mucosa of *Neotragus* and *Mouflon* (Ungulata). In these forms the lumina of arterioles open *directly*, through a valvular mechanism in the wall of the arteriole, into large venous blood spaces without the intervention of a specialized connecting channel.

The PRESIDENT commented on the large size of the blood spaces in the human nasal mucosa, and Professor LE GROS CLARK suggested that there might be some relationship between the blood vascular channels of the mucosa and the olfactory nerves.

Dr G. H. BOURNE read a paper on 'Alkaline phosphatase and vitamin C deficiency in regeneration of skull bones'.

Holes, 1 mm. in diameter, were bored in the parietal bones of the skulls of guinea-pigs and

the holes permitted to heal for periods varying from 24 hr. to 2 weeks. Undecalcified sections were cut through the skull passing through the healing hole. Alkaline phosphatase (Gomori's method) preparations were made of these sections. Some of the animals were daily injected intraperitoneally with trypan blue for a week preceding operation and until autopsy. These experiments were also repeated with guinea-pigs which had been on a scorbutic diet for a week prior to operation.

The results showed that there was an accumulation of cells resembling polymorph leucocytes, which gave an intense positive phosphatase reaction, at the site of injury. Osteoblast-like cells and cells which appeared to be forming capillary vessels were also present. They were phosphatase positive. Macrophages were also present in large numbers in the injured area; they gave no phosphatase reaction.

At 3 days there was a considerable decrease in the number of polymorph-like cells in the injured area, but numerous phosphatase-positive fibres and some positive capillaries were present. At 1 week massive concentrations of phosphatase in the injured area heralded the onset of the trabeculae.

Guinea-pigs on a scorbutic diet showed a reduction not only in the production of fibres but in the number of polymorph-like cells and macrophages present in the injured area. The ability of those polymorph-like cells which were present in the injured area to give the phosphatase reaction was unaffected by the scorbutic condition.

Dr WYBURN stated that bone continued to form even after treatment with cyanide which is presumed to inactivate phosphatase. Dr BOURNE replied that he was not directly concerned with this problem and he did not deny or assent that phosphatase was concerned in bone formation.

Dr A. SLESSOR and Dr G. M. WYBURN read a paper on 'Bone formation'.

The occurrence of bone formation in the host tissues (guinea-pig), following the presence of bone homografts in the subcutaneous tissues for 5–6 days, was previously reported (Wyburn & Bacsich). Bone homografts consisting of the femoral epiphysis (bone and cartilage) were implanted and maintained in the subcutaneous tissues of guinea-pigs for 3 weeks. There was no evidence of bone formation by the tissues of the host.

Bone homografts (pieces of shaft of femur) were implanted into the flexor muscles of the hind limbs of rabbits. After 4 weeks there was no evidence of bone formation by the host tissues.

Blum (1944) asserts that he obtained formation of bone in rabbit muscle after the intramuscular injection of phosphatase. His experiments were repeated and, in addition, pellets of phosphatase were implanted intramuscularly and left for 4–5 weeks. There was no evidence of bone formation.

Dr BOURNE, in support of the evidence presented, stated that he had introduced extracted rabbit phosphatase into holes drilled in rabbit femora; in others he had covered similar holes with filter-paper containing rabbit phosphatase; in others he had implanted phosphatase in powder form and on filter-paper subcutaneously; he had also implanted pieces of alcohol-fixed urinary bladder and gall bladder into and over holes in rabbit femora and in no case was there any apparent action on bone formation.

Dr P. BACSICH and Professor W. J. HAMILTON read a paper on 'Supravital staining experiments on the tubal ova of the rabbit'.

1/10,000 aqu. solution of toluidin-blue was applied as supravital stain to living tubal ova of the rabbit. Sections of tubal eggs were also stained with toluidin-blue.

The 'albuminous' coat gradually deposited on the rabbit's ovum as it passes down the tube gave an intense metachromatic staining in all of the experiments, thus indicating that it is mainly composed of muco-proteins. Staining results also suggest in the tubal eggs the presence of a slow enzymatic hydrolysis of this muco-protein coat immediately outside the zona pellucida, and a more rapid hydrolysis contemporary with, and probably mainly responsible for, the rapid expansion of the blastocyst when it reaches the uterine cavity.

These supravital staining experiments, probably the first ever carried out on living mammalian ova, also revealed the existence in the tubes of large muco-protein spherules, structures closely resembling those found by Hartman (1916 and 1919) in the opossum.

The significance of these findings was discussed.

FEBRUARY 1948

An ordinary meeting of the Society, for the Session 1947–48, was held on Friday, 20 February 1948, in the Department of Anatomy, The Medical School, Hospitals Centre, Birmingham, the President (Professor A. B. APPLETON) in the Chair.

Dr T. L. KROHN and Professor S. ZUCKERMAN read a paper entitled 'Stimulation of the seminiferous epithelium of immature monkeys with androgens'.

Androgens stimulate and oestrogens depress the germinal tissues of immature female monkeys. In view of this observation it was of interest to reinvestigate the effects of the same hormones on the immature monkey testis. It is confirmed that oestrogens depress the size and activity of the seminiferous tubules. Androgens, on the other hand, have a stimulating effect, although, as previously reported, sperm formation has not been observed in our experiments. Our new observations can be related to comparable findings on rats, and on hypophysectomized mature monkeys.

Professor ZUCKERMAN pointed out that the same material on which he and Dr KROHN were reporting had been investigated by him alone earlier, in order to discover whether androgen stimulated spermatogenesis in immature monkeys. The results of the earlier investigations were negative, but no attention had been paid to detailed changes in the first stages of germinal development.

Professor BOYD congratulated the authors on their findings and referred to the work of Töndury on the effect of androgens on the speed of cleavage in growing eggs.

Dr A. P. D. THOMSON and Professor S. ZUCKERMAN read a paper on 'The vascular and nervous connexions of the retina concerned in the response of the gonads to light'.

Previous experiments designed to determine the optic pathways along which light stimulation of the retina affects the gonadotrophic potency of the ferret pituitary have led to equivocal results. The problem is being reinvestigated in order to discover first, whether the initial stimulus is a nervous one, and second, whether it can be elicited with the pituitary transplanted away from the sella turcica. Four groups of animals have been used: (*a*) animals in which only the optic nerve fibres have been divided, and in which the central artery of the retina, and the long and short ciliary vessels and nerves are left intact; (*b*) animals in which the optic fibres are not disturbed, but all these other structures are crushed; (*c*) animals in which both sets of connexions are divided (as in all previous corresponding experiments in which the 'optic nerves' have been divided); and (*d*) controls. The results of this experiment show that the gonadotrophic response is dependent on the integrity of the optic nerve fibres themselves.

Professor BOYD inquired as to the presence or absence of myelinated and non-myelinated fibres in the optic nerve. Dr THOMSON replied that no histological examination had been made.

Dr BOURNE asked if the authors had any information regarding the effect of light on the reproductive cycle of monkeys that do not have a long seasonal period of anoestrus. Professor ZUCKERMAN replied that there was no information, to the best of his knowledge, about the influence of light on the reproductive cycle in monkeys, but that it had been shown by Broman that in the rat optic enucleation causes a marked delay in the various phenomena characteristic of normal growth and sexual maturity.

Dr GLEES inquired concerning the difference between 'optic nerve fibre cut' and 'optic nerve cut'. Dr THOMSON answered that in the former the sheath of the optic nerve and all the contents of the sheath are damaged as little as possible, and only the fibres of the optic nerve are severed. By this means only the central neural connexions between the eye and the

brain are involved and the circulatory connexions are preserved. In the case of optic nerve cut the sheath and its contents along with the optic nerve fibres are cut and no blood or nervous connexions between the retina and the brain are left.

Professor YOFFEY inquired about the possibility of degenerative changes following section of the optic nerve. Dr THOMSON replied that while no histological examination had been made he expected to find that a degeneration of the ganglion cell layer of the retina had occurred, and he cited cases in man in which this had been noted following certain haemorrhages, e.g. post-partum, melaena, etc.

Professor GOLDBY remarked that he did not consider that Jefferson's negative findings in the ferret, so far as hypothalamic connexions of the optic nerve were concerned, were entirely convincing. He had himself observed aberrant optic fibres in the sheep which, in some cases, run towards the pre- and supra-optic regions. These fibres were not traced to their termination, and it may be that they return to the optic tract although they have not been seen to do so. In his opinion the question of hypothalamic optic connexions was still an open one.

The PRESIDENT congratulated the authors on their technical skill during these delicate operations on the optic nerve.

Dr I. C. MICHAELSON (introduced by Professor W. J. HAMILTON) read a paper on 'Vascular morphogenesis in the retina of the cat'.

A study of injected retinae of foetal and adult cats shows the following:

(1) Vessel growth in the retina of the cat is by a process of budding from pre-existing vessels. No evidence could be found of vascular differentiation from local cells.

(2) The formation of retinal capillaries is pre-eminently a function of the retinal veins. Only the arteriae afferentes appear to originate from arteries.

(3) If vein and artery are close to each other, growth takes place initially from the side of the vein remote from the neighbouring artery.

(4) The spread of capillary growth towards an artery extends for only a certain distance, leaving a well-marked capillary-free space around the arteries similar to that present in other mammalian retinae such as those of Man, dog, rat and pig.

(5) These anatomical facts are clearly associated with each other. Considered as a group they suggest the presence of a factor or factors which affect the growth of retinal blood vessels.

(6) The present ontogenetic study shows that the bulk of the capillary system in the retina of the cat can be considered as part of the venous system. The arterial system is shown to be supra-capillary in origin.

Dr BOURNE asked if the growth of the regional capillaries might be due to the action of inhibitory substances released. Dr MICHAELSON replied that this was a possibility. Dr BRANDT commented that the author's injections showed only those capillaries whose calibre allowed the injection mass to enter. The early solid endothelial outgrowths from the arteries cannot be demonstrated in this way. He suggested a further histological examination.

Dr WYBURN remarked that environmental differences might affect the new growth of the capillary network.

Dr B. H. DAWSON read a paper on 'The blood supply of the optic chiasma'.

Since neurologists at the present time favour chiasmal pressure ischaemia as the probable cause of visual field defects in patients with tumours in the chiasmal region, it is necessary to establish the exact blood supply of the optic chiasma.

The details given of the chiasmal 'Epineural Vessels' were based on dissections of 230 fresh P.M. brains; and in order to avoid tearing fragile vessels the greater part of the sphenoid was removed with the brain *en bloc*.

The intimacy of association between the blood vessels of the chiasma, and those of the hypophysis and hypothalamus was described and illustrated and another arterial anastomotic system with the circulus arteriosus was demonstrated.

The foramina through which the vessels entered the substance of the chiasma were clearly shown, as was also the supraclinoid hypophyseal system and its distribution both along the infundibulum and over the inferior surface of the optic chiasma.

Seen clinging to the anterior border of the chiasma was the prechiasmal arcade joined by vessels from the ophthalmic, internal carotid, and anterior cerebral arteries.

The many small vessels from the proximal part of the anterior cerebral artery which provides a rich supply to the supraoptic nuclear region were shown and the high vascularity of this region noted.

Professor ZUCKERMAN asked for further information about the intimacy of association between the vessels of the chiasma and those of the hypophysis and hypothalamus.

Dr DAWSON replied that he had found a very close connexion between the blood vessels of the chiasma, hypophysis and hypothalamus and mentioned how very difficult it was to keep the injection material well localized to any one of these areas. Even with the profuse application of Cushing's haemostatic clips both before and during injection of the coloured gelatine it had been found difficult to fill only the vessels of one of those three systems.

Professor HAMILTON remarked that the pressure required to obliterate the complex system of vessels shown would need to be remarkably high, and wondered against which unyielding structure these chiasmal vessels might be compressed. Would not the fibres of the chiasma be damaged before their blood vessels were obliterated?

Dr DAWSON said that the whole question of pressure ischaemia and nerve conduction interference was very unsettled and that he was attempting to elucidate these problems.

Dr MICHAELSON favoured the vascular compression mechanism in view of the rapidity of recovery of visual field defects following decompression.

Professor YOUNG remarked that the vascular pattern on the chiasma was very like that of peripheral nerve, and inquired whether longitudinal anastomoses had been observed.

Dr DAWSON replied that he had been impressed with the similarity in pattern between the vascularization of the chiasma and the peripheral nerves. He could demonstrate the epineural longitudinal vessels in his photographs.

Dr W. C. OSMAN HILL read a paper on the 'Blood supply of the human caecum'.

Standard descriptions are based upon figures by Jonnesco (in Poirier's *Anatomie humaine*) and subsume a symmetrical arrangement of anterior and posterior caecal arteries. This is not so in lower Primates, with one exception (*Ateles*), and hardly to be expected in Man in view of difference in size of the two vessels. Preparations were shown to indicate that the anterior artery has a relatively limited distribution on the ventral aspect of the basal part of the caecum and on an area opposite the ileal entrance. The rest of the caecum, including the ventral aspect of its fundus, is supplied by branches from the posterior vessel, one proceeding ventral to the root of the appendix. The appendicular artery is independent of both caecal trunks in Hominoidea. A specimen of the rare anomaly of substitution of the dextral for the sinistral supply to the appendix with associated peritoneal anomalies was demonstrated.

Dr BRANDT inquired if any study had been made on the vascularity of the developing human caecum and Dr HILL replied in the negative.

Professor WEST referred to the position of the meso-appendix and asked about the factors concerned in its final disposition on either the ventral or dorsal side of the terminal portion of the ilium, and whether this was in any way associated with the rotation of the gut. Dr HILL replied that usually the mesoappendix was sinistral, but that dextral substitution occurred occasionally in man and orang and was normal in *Lagothrix*, and about equally common with sinistral arrangement in *Aotes*. The factors deciding this are unknown.

Dr GLEES inquired whether the vascular arrangements were different in the retrocaecal appendix and was informed that topographical relations had no effect in determining the arterial supply in Man.

Professor KIRK remarked that in removal of the appendix, bleeding from its base is sometimes observed even after ligature of the appendicular artery. He considered that this was from a branch of the anterior caecal artery. In view of the fact that the appendix developmentally is part of the caecal wall this is not surprising.

The PRESIDENT commented on the bloodless fold of Treves, and Dr HILL, in reply, stated that this fold was much more extensive in the lower primates and was primitively anangious, but that occasionally both in monkeys and Man a recurrent vessel entered it from its dorsal edge derived from the morphologically sinistral side.

Dr E. W. WALLS read a paper on 'An investigation into the regenerative capacity of mammalian heart muscle'.

It has been recently demonstrated that striped muscle in rabbits and rats possesses considerable power of regeneration after injury (Le Gros Clark, 1946). Moreover, King (1940) described a case of fatal cardiac injury in Man in which he claimed to have found clear histological evidence of regeneration of myocardial fibres. Accordingly, it was decided to investigate the response of rabbit's cardiac muscle to injury. Lesions were made in the lower third of the ventricular myocardium by the application of the head of a nail heated to a dull red heat, and the animals were allowed to survive for periods ranging from 3 days to 1 month. The injured areas were then studied for evidence of regeneration of cardiac muscle fibres. No such evidence has been obtained. It is thought that the type of lesion used is possibly too severe, producing too great a reaction in the adjacent tissue. Burning was selected as the method as it had previously been used successfully (Thomas & Harrison, 1944) for functional recovery tests in rats, and because it is difficult by crushing to produce comparable lesions in rapidly beating hearts.

Dr E. FRANCIS asked whether Dr Walls had seen anything in his preparations that would substantiate the claim of Retzer that ordinary heart muscle developed from Purkinje fibres. The reply was in the negative.

Dr A. LASNITZKI remarked that embryonic heart muscle shows in tissue culture pronounced regenerative growth. It would be of interest to know whether, and to what extent, tissue culture experiments have been carried out with heart muscle from adult animals, since the results might have an important bearing on the question of the actual potency for regeneration still existent in myocardial fibres.

Dr FIELD asked whether Dr WALLS believed that intercalated discs did in fact exist in fresh well-fixed, celloidin cut sections of heart. The reply was in the affirmative.

Dr H. A. MEYLING (introduced by Professor R. D. LOCKHART) read a paper on 'The structure of autonomic nervous tissue in the right atrium of the heart in some mammals'.

By using the Bielschowsky-Gros silver impregnation method, a dense network of 'autonomic interstitial cells of Cajal' can be observed in the loose connective tissues of epicardium and endocardium. In the endocardium they are closely connected to the Purkinje cells (which, in collaboration with Ter Borg (1939), were found forming a dense network beneath the endothelial lining covering the musculi pectinati, and which are neither connected with the S.A. node nor the A.V. node but pass on all sides into the ordinary muscle fibres of the right atrium) and also to the smooth muscle cells which in silver preparations can be seen scattered in the loose connective tissues of the endocardium.

Again, in both the S.A. and A.V. nodes, a net of 'interstitial cells', especially well marked in the horse, is found as the most peripheral extension of the autonomic nervous system. They form a lace-work around the small nodal fibres, their branches running alongside these fibres, and in some of them Nissl-substance is found by using the selective stain of Einarson. The difficulty of showing Nissl-substance in the 'interstitial cells' may be due to the functional state of the cell at the moment of fixation, for Einarson has pointed out that during activity the ganglion cell loses its Nissl-substance. Changes in the staining of the nucleus of the 'interstitial cells' (with cap formation on one pole) were found comparable with those seen by Einarson in the ganglion cell, but further experimental research is needed for the interstitial cells, similar to that by Levi, Einarson and others for the ganglion cells.

This network of 'interstitial cells' ought to be identified with the 'sympathetic groundplexus' of Boeke, and the 'pre-terminal reticulum' of Reiser-Stohr. Leeuwe (1937) has pointed out that these 'interstitial cells' are small anastomosing ganglion cells, containing Nissl-substance. In the carotid sinus and the wall of the aorta, I found (1938) that these 'autonomic interstitial cells' contain Nissl-substance, and are connected with the end

ramifications of the post-ganglionic fibres in an apparently synaptic manner, because their neuro-fibrillar network remains intact after experimental degeneration of the post-ganglionic fibres.

Accordingly, the sensory nerve endings as described by Smirnow, Dogiel and Woollard in endocardium and epicardium, and by Nonidez and Pannier in the sinuatrial node, are probably the end ramifications of the post-ganglionic fibres forming synapses on the network of the 'autonomic interstitial cells', and the latter are identical with the star-shaped cells and the branching cells described respectively by Dogiel and Woollard in their methylene-blue preparations. Nonidez and Pannier, the former denying the nervous character of the peripheral nervous network, could not in fact demonstrate the 'interstitial cells' as a system, by the silver method they employed.

This conception of the structure of the peripheral autonomic nervous system was obtained in the carotid sinus (1938) by combining the results of silver impregnation and Nissl staining with the vital methylene-blue staining. Now the right atrium has been treated similarly, using vital methylene-blue staining in the modifications given by Schabadasch.

Dr FIELD remarked that the author's description of the large amount of nervous tissue in the heart was of great interest for any theory of cardiac action. Much nervous tissue accompanies the Bundle of His in many animals, and it was of interest to recall the statement of Cohn and Trendelenburg in 1910 that experimental proof of muscular as against nervous conduction in the Bundle had never been achieved up to that time, nor indeed has it since.

Professor BOYD suggested that the cells demonstrated by Dr MEYLING as nerve cells were in reality connective tissue cells.

Dr BRANDT remarked that silver nitrate is reduced in the tissues by very different substances, and we have no methods to hand to prove the metabolism of the tissue responsible for the reduction of silver. Boeke found intrasarcoplasmic endings in the muscle fibres of the heart which differ from the motor endings the author had shown.

Dr MEYLING agreed that sections impregnated with silver need careful interpretation, but with experience it is not difficult to differentiate between connective tissue and nervous structures. In the modification of the silver technique by Gros, by changing the amount of ammonia, the impregnation of connective tissue cells can be entirely suppressed. He had seen autonomic interstitial cells in epi- and endocardium only in places where end branches of afferent or post-ganglionic branches were present, and in the preparations of the A.V. and S.A. nodes, they appeared only in nodal tissue and not in adjoining musculature. If they were connective tissue cells, these cells would be impregnated everywhere in the connective tissue and in the heart musculature. Moreover, the nervous nature of the autonomic interstitial cells is not merely based on the fact that they are impregnated with silver but on the typical nervous structure they show in methylene-blue preparations.

Dr FRANCIS asked whether interstitial cells were found also in the ventricles, and if so were they similar to the interstitial cells of the enteric plexus. Dr MEYLING replied that they occur everywhere in the heart and that they react to the technique employed in a similar way to those of the enteric plexus.

Dr W. A. FELL read a paper entitled 'The effect of sympathectomy on the size of Haversian canals in the cat'.

In order to induce an increased flow of blood through the bones of the lower limb the lumbar portion of the sympathetic trunk of one side was removed in two cats. Examination of sections of the control and treated tibiae 1 and 10 weeks respectively after operation did not show any gross decalcification on the treated side. The size of the Haversian canals in the compacta of the shaft was then estimated by projection and drawing of their outlines at a fixed magnification. The area of these canal outlines was then estimated by Schmalhausen's method, i.e. the outlines were transferred to Bristol board, cut out of the board and weighed. By this means a series of estimates of size were obtained directly proportional to the sizes of the individual canals. Statistical analysis of these figures shows that there is a definite and significant difference between treated and untreated sides (the treated side

being greater), and therefore that increased blood-flow on the treated side has caused an increase of canal diameter through absorption of the wall. This is attributed to increased blood-flow and not to any increased activity of the osteoclasts, whose numbers do not show any change.

Leriche and Policard's hypothesis, that increased vascularity of bone produces decalcification, is held to be proved, though the biochemical mechanism of this process remains obscure and offers further problems for investigation.

Dr BOURNE remarked that he was under the impression that the work of Leriche showed that if the bone of an animal was broken the phosphatase content of the whole skeleton increased and that this could mean either absorption or deposition of bone, since the enzyme is concerned in both processes. Dr FELL agreed that this was so.

Dr PRITCHARD said that he was surprised that the decalcification was not more extensive in view of the striking changes which may follow lesions involving the sympathetic in clinical practice. He suggested that the effects on bone of active and passive hyperaemia ought to be distinguished carefully as the former predisposed to calcification while the latter stimulated new bone formation.

Dr FIELD inquired if the sections were taken at comparable levels and whether they were cut at a slant. Dr FELL replied that the pieces from the right and left tibial shafts were taken from the same portions of the bones, and the serial sections selected for study from these pieces were from the same level. Great care was taken in all cases to get the plane of section at right angles to the long axis of the bone. He did not think that the amount of slant ever exceeded 5° and even that amount is readily seen in adjusting the block.

Dr F. FYFE inquired whether a planimeter had been used to measure the areas of the canals on a much enlarged image of the slides. Dr FELL replied that he had used this instrument but did not find it accurate enough, some of the smaller canals giving no reading.

Dr G. T. ASHLEY read a paper entitled 'On the insertion of the pectoralis major'.

Fourteen cadavers and four foetuses were examined to determine (*a*) whether the tendon is bilaminar or trilaminar; (*b*) which of the fibres actually twist before reaching their insertion; (*c*) through which lamina the various fibres are inserted.

In all cases the tendon was found to be bilaminar. This is contrary to the view expressed by Grant of Toronto.

The only fibres involved in twisting are the SUPERFICIAL fibres from the lower sternocostal and abdominal elements, plus, occasionally, a few superficial fibres from the manubrial and clavicular portions. These fibres join an aponeurosis on the inferior border of the muscle 1–2 in. from the bone before twisting backwards, upwards and then laterally, fanning out to form the posterior lamina of the tendon.

The anterior lamina is formed mainly of tendon derived from the manubrial portion. The majority of fibres of the clavicular portion are inserted into the front of this lamina, and a definite, though variable, contribution of deep fibres from the lateral edge of the body of the sternum and adjacent 3rd, 4th, 5th and (?) 6th costal cartilages passes without twisting to join the posterior aspect of the anterior lamina. These fibres are not described in the text-books.

From the sixth rib a bundle of non-twisting fibres passes upwards and laterally to join either the anterior aspect of the posterior lamina or the posterior aspect of the anterior lamina. These fibres are not described in the text-books.

The PRESIDENT suggested that the tendon of the muscle may be considered to be multilaminar some 2 in. from the line of insertion. Dr ASHLEY replied that the non-twisting part of the muscle is multilaminar at this point, but that as the muscle fibres become aponeurotic they fuse with those of adjacent bundles to form the accepted anterior and posterior laminae, and it is only rarely that multilamination of the tendon is apparent unless sharp-knife dissection is used.

Dr BRANDT raised a query on the analogy of the twisting of pectoralis major and the arrangement of the teres major and latissimus dorsi tendons. Dr ASHLEY replied that the question of twisting in other muscles was part of a wider problem under study.

Dr A. C. Allison read a paper on 'The histology of the stomach in the Insectivora'.

In three unrelated Insectivora, *Elephantulus myurus*, *Suncus orangiae* and *Eremitalpa granti*, the stomach has been studied. While the gross structure differs greatly in these three genera, microscopical examination shows that the same type of glandular organization is present in all cases. This organization is simple and generalized. It is suggested that in the gastric glands there may be distinguished a gradient of organization which appears to be related to the degree of differentiation of the cells in the glandular epithelium.

Professors Zuckerman and Boyd raised the question of the phylogeny of *Elephantulus*. Dr Allison replied that the family Macroscelididae, of which *Elephantulus* is a member, had been grouped by the older systematists in a suborder of the Insectivora, referred to as the Menotyphla, which exhibited distinct Primate affinities. Though later work had cast some doubt upon a close relationship between the Macroscelididae and the Primates, a recent detailed osteological study had reaffirmed such a relationship. Moreover, the presence of a menstrual cycle had been demonstrated in *Elephantulus*. Whatever the systematic position might be, the stomach in *Elephantulus* is very similar in form and relationships to the stomachs of the lower Primates.

To a question by Professor Boyd concerning the nature of the ectopic mucosa in Meckel's diverticulum, Dr Allison replied that as far as ectopic gastric mucosa in this situation was concerned, preparations examined personally had glandular tubules composed of mucoid cells, as in ectopic gastric mucosa in oesophageal 'erosions'.

Dr D. C. Sinclair read a paper on 'The intervertebral ligaments as a source of segmental pain'.

It has been maintained by Kellgren that the posterior intervertebral ligaments constitute an important source of referred pain in cases of sciatica and pain in the back. This contention is based on the results of injecting hypertonic saline in the region of the mid-line of the back without verifying the position of the point of the needle radiographically. In this work, little attention was paid to the anatomy of the region into which the injections were made, and as a result it was assumed that the findings were due to the stimulation of the interspinous ligaments. These observations have been repeated under radiographic control, and the findings are inconsistent with the hypothesis that the resulting referred pain is due to the stimulation of the ligaments, while the evidence strongly suggests that it arises from the direct stimulation of nerve trunks in the vicinity, such as the medial branches of the posterior primary divisions of the spinal nerves. Accordingly, the significance of lesions of the posterior intervertebral ligaments in the production of segmental pain may be regarded as not yet established.

Dr Wyburn made a congratulatory comment on the fact that an alternative cause of low back pain had been suggested other than the inevitable prolapsed disc. He remarked also on the widespread nature of pain resulting from subcutaneous injections of hypertonic saline, from personal experience.

Professor Boyd offering his congratulations remarked on the excellent demonstration that Anatomy was still the basis for clinical science!

APRIL 1948

An ordinary meeting of the Society, for the Session 1947–48, was held on Friday, 30 April 1948, in the Rooms of the Zoological Society of London, Regent's Park, the President (Professor A. B. Appleton) in the Chair.

Dr R. E. Rewell read a paper on 'The caecum of the Hyracoidea'.

The intestines of this Order were described by Owen (1832), Flower (1872) and Beddard (1908). The ileum ends in a dilated sac in the left iliac fossa. This is sacculated and two taeniae run, one on to the anterior and one on to the posterior surface from the colon. Each is associated with an artery which raises a small ridge of peritoneum. They are of

equal size. A straight length of gut with longitudinal muscle layer and about 20 cm. long, runs from this sac and turns cephalad in the right iliac region. In *Dendrohyrax* a small knuckle protrudes from this on the antimesenteric border, on to which a small artery runs on the posterior aspect. There is no anterior artery to it. I have not found this diverticulum in the genus *Procavia*. An anangious fold of peritoneum lies in the angle between the proximal dilated sac and the gut leading from it. Where this gut turns cephalad, two symmetrical diverticula arise from the antimesenteric border. They are relatively large, are directed caudad and end in a point. No peritoneal folds are associated with these and several small arteries run on to each from the mesentery without raising ridges.

Earlier authors considered the first sac to represent the true caecum, but Chalmers Mitchell (1916) favoured the paired diverticula and compared them to the structures seen in birds, in *Trichechus* and *Cyclopes*. Since the proximal sac lay on the left side and its anangious fold lay on the opposite side to the usual, he did not consider it to represent a caecum.

However, only the proximal sac has arteries on the morphologically dextral and sinistral aspects, while all caeca that I have examined have such, from lizards, such as *Varanus* and *Uromastyx*, up to the Primates. Moreover, this sac only is sacculated. On these grounds it may well represent the true caecum.

The PRESIDENT, congratulating the author, asked why the blood vascular pattern was stressed at the expense of other morphological features. Dr REWELL replied that this was deliberate and he wished to stress its importance in connexion with the caecum of all mammals. He agreed with the President that it was not the sort of feature usually considered to be of importance, but he had found this distribution of arteries in all the mammalian, and even reptilian caeca examined. It is, for instance, universal throughout the Primates.

To Professor YOFFEY, who inquired as to the presence or absence of a caecal sphincter, Dr REWELL replied that the material was not well enough preserved for the histological study of this or any other feature.

Dr HINDLE asked if there was any peculiarity in the rodent caecum which might throw light on caecal morphology. Dr REWELL replied that he had examined a number of rodent caeca and they were all relatively simple and quite unlike that of the Hyracoidea, however complex their colons.

Dr G. H. BOURNE read a paper entitled 'Some observations on the mammalian adrenal gland'.

Over a period of some seventeen years histological examinations have been made of the adrenals of large numbers of Marsupials and other Mammals and a detailed list of the observations of other workers on the mammalian adrenal has been made. Details of the structure of the adrenals of some 250 species are thus available. The present paper consists of a summary of the main points which have emerged from this study.

These are:

(1) The Monotreme adrenal differs appreciably from the adrenals of the Marsupials and the Eutheria and resembles those of the Reptilia.

(2) All Marsupials show an adrenal with the typical mammalian arrangement of cortex and medulla.

(3) There is a remarkable constancy of adrenal structure throughout the whole mammalian kingdom, from the lowest Marsupials up to Man.

(4) The cortical zones cannot be distinguished in the adrenals of all species.

(5) Lipoid droplets are found almost invariably in some of the cells of the adrenal cortex of all species, including the Monotremes.

(6) Vitamin C (as demonstrated by the acetic-acid silver nitrate technique) appears to be present in high concentration in the adrenal cortex of Monotremes and some twenty-four other species of Mammals to which this test has been applied.

(7) A black pigment which appears to be melanin, and a yellow lipochrome pigment are present in the adrenals of a large number of species of Mammals.

(8) Some representative of the 'X' or juvenile zone appears to be present in the adrenals

of a large number of species of Mammals. First described by Howard-Miller in the mouse, representatives of the zone have been found in other rodents, Insectivores, Ungulates, eleven species of Marsupials, bats and one species of monkey.

Professor YOFFEY inquired if there was any species in which a constant amount of islet tissue occurred; if so, such might be useful in experimental studies. Dr BOURNE replied that it was not sufficiently constant in any one species to be significant. He agreed with a further comment by Professor YOFFEY that it would be useful to secure exact data on cortico-medullary ratios, but relatively few species had been studied in this respect.

Mr N. CAPENER read a paper on 'Physiological splintage'.

In the treatment of diseases and injuries of the locomotor system, support of joints in physiological positions is a recognized feature. Apparatus which will control the position, yet permit movement, and which may assist in the replacement of deficient muscles, is a well-known method applied in the treatment of peripheral nerve injuries. Apparatus using springs and designed to facilitate the recovery of function by providing reflex stimulation through the proprioceptive system, has been advanced, particularly in the treatment of the hand, in the type of apparatus called 'Lively Splints' developed at Exeter.

Dr WHILLIS said that such splintage was a distinct advantage particularly in providing greater elasticity in central palmar control. In reply Mr CAPENER said that there was a common misconception that splints are devices to immobilize parts of the body, and that his main purpose was to interest members in a problem of applied anatomy and physiology in the hope that it would lead to co-operative effort by clinicians and anatomists to further study along these lines. The splints for the hand, while leaving free the greater part of the important tactile area do, by providing spring balance substitution for deficient muscles, aid in the normal activation of peripheral reflexes and diminish inhibitory activity which we may expect to arise in muscles ineffectively opposed.

Drs C. D. CHANNELL and J. WHILLIS presented a communication on 'The effect of ulnar nerve block at the level of the pisiform bone on movements of the ring finger'.

When the subject's metacarpo-phalangeal joint is fixed by the observer, extension of the interphalangeal joints is difficult and the subject complains of discomfort over the dorsum of the metacarpal head. The taut extensor tends to become displaced to one or other side due to the loss of the stabilizing effect of the muscles inserted into the extensor hood. The discomfort and loss of power can be explained on the assumption that the lumbrical does not produce the necessary relaxation of the flexor to allow the movement to occur.

When the first phalanx is allowed to move freely, and attempts at opening the hand are made, most of the power of the extensor is expended on the first phalanx which becomes hyper-extended owing to the loss of the stabilizing effect of the third lumbrical and the third palmar and fourth dorsal interosseus.

Attempts at the 'needle-threading' movement (flexion of the metacarpo-phalangeal and extension of the inter-phalangeal joints), using the thumb and the ring finger, result in ulnar deviation of the ring finger due to loss of the opposing action of the lumbrical. The metacarpo-phalangeal joint is hyper-extended, the first inter-phalangeal joint fully flexed and the second inter-phalangeal hyper-extended. The correct movement of the inter-phalangeal joints can be restored by passive flexion of the first phalanx.

Professor YOFFEY asked what the role of the first dorsal interosseous muscle was in gripping between the index finger and the thumb. Dr WHILLIS replied that with the fixed index it could not produce abduction but probably rotated the index and fixed the thumb metacarpal.

Dr J. J. PRITCHARD read a paper on 'Alkaline phosphatase in the uterine epithelium of the rat'.

The distribution of alkaline phosphatase in the uterine epithelium of the rat has been studied in the immature animal, during the stages of the oestrous cycle, in anoestrus, in

pregnancy and after bilateral ovariectomy, and following the administration of stilboestrol and progesterone.

The findings suggest that oestrogens stimulate the manufacture of the enzyme and its secretion into the uterine lumen.

Dr BOURNE remarked that since the production of glycogen is believed to follow the path of splitting off of phosphate from hexosediphosphate and subsequent condensation of hexose molecules, and since phosphatase appears to be an enzyme which could split off phosphate, it would be interesting to know whether Dr Pritchard had observed any parallelism between the distribution of glycogen and phosphatase in his preparations. Dr PRITCHARD replied that he had no glycogen preparations, and he thought that there was no evidence that phosphatase played any part in the synthesis of glycogen.

Dr FIELD remarked that there was no such parallelism in heart muscle, and added that here there was abundant glycogen and practically no phosphatase. Dr BOURNE added that phosphatase was not the only enzyme which could split hexosediphosphate.

Dr R. J. HARRISON read a paper entitled 'Observations on the ovary and reproductive tract of the Ca'aing whale, *Globicephala melaena*'.

The material was obtained from a school of 300 Ca'aing whales killed at Torshavn, Faroe Islands, in March 1947, and was made available through the kind offices of Dr Carl Bech, Chief Veterinary Officer, Faeroe Islands. Ovaries were removed from a total of thirty-eight females and entire reproductive tracts from twelve females and one 42 cm. foetus. The specimens were obtained from six immature females, twelve maturing females, six females, whose ovaries contained one retrogressing corpus luteum, and fourteen adult females, the ovaries of which contained two or more retrogressing corpora lutea.

An account of the anatomy of the reproductive tract is given, and the general structure is shown to be essentially similar to that described for *Phocaena* by Meek (1918). The changes occurring in the ovaries, uterine tubes, uterine horns and uterus during growth are briefly described. A marked thecal gland is present around the actively growing follicles found in the ovaries removed from maturing whales.

The ovaries from five whales contained either recently ruptured follicles or developing corpora lutea. The histological appearances of these corpora lutea are described: many of the markedly vacuolated luteal cells become multi-nucleated during the later stages. The old corpora lutea apparently persist for a longer time in the ovaries of *Globicephala* than in those of other Eutheria. However, the appearance indicates that the corpora lutea do eventually disappear, and do not persist throughout life in a degenerated state as is said to occur in the Balaenopteridae (Peters, 1939).

Dr OSMAN HILL commented on the curious evagination in the cervical canal and inquired if the author had studied the histology of this region. Dr HARRISON replied that this study was being undertaken, and Dr BAXTER remarked that it might throw light on the precise extent of cervix and vagina in these specimens.

Drs J. DAVIES and D. V. DAVIES read a paper on 'The mesonephros of the sheep'.

The cranial third of the Wolffian body in the sheep is occupied by a peculiar glomerular structure called for convenience the 'giant glomerulus'. Here is a more or less continuous glomerular cavity, drained by about twenty tubules, and traversed by a series of fenestrated vascular plates. This giant glomerulus has been previously described briefly by Bremer in sheep, cow and deer, but he did not explain the manner of its development correctly. Caudally the giant glomerulus is in series, through a short transitional zone, with a zone of some forty or more ordinary Malpighian bodies similar to those found in other mammals. The study of the development of the various zones in the sheep's Wolffian body provides much information as to the factors determining the form and disposition of both the glomeruli and tubules in the vertebrates generally. Furthermore, the giant glomerulus provides a useful landmark for the study of the degeneration of the mesonephros and of the site of the urogenital union. No massive degeneration of the cranial end of the mesonephros occurs in the sheep and the urogenital union occurs through approximately the 6th–12th

tubules. This is in contrast to the description by Felix of the human and accords well with that of most other workers on the mammalian mesonephros. The cellular processes involved in the degeneration are similar in all mammals.

Dr J. DAVIES read a paper on 'The arterial blood supply of the Wolffian body in the sheep'.

The arterial blood supply of the sheep mesonephros has been studied by means of injections with indian ink. The vascularization of this organ is shown in relation, first, with the so-called 'giant glomerulus' and secondly with the group of ordinary Malpighian bodies caudal to it. The former comes to be supplied by a single large vessel from the aorta and appears in this light as an early functional unit. The ordinary Malpighian bodies do not develop their connexions with the aorta until a relatively late stage, when the giant glomerulus is already beginning to degenerate. MacCallum's findings (1902) of multiple efferent vessels in the glomeruli of the pig mesonephros is confirmed for the sheep, and the manner of their development and connexions is studied.

Professor J. P. HILL congratulated the authors of the above two papers and commented on the differentiation of the glomerular capillaries in a site quite independent of the dorsal aorta. This had been missed by the majority of previous workers.

Drs G. N. C. CRAWFORD and R. BARER read a paper on 'The action of fixatives on living cells as studied by phase-contrast microscopy'.

The actions of various simple fixatives on living cells teased from the testis of *Salamandra maculosa* have been studied by phase-contrast microscopy and photography. This enables the cells to be examined during and after the process of fixation without further treatment or staining. Other vertebrate cells in addition to those of salamanders were also studied but in less detail. Of the substances studied, formalin has proved to be of particular interest. The general actions are:

(1) An initial shrinkage and distortion of the cell, varying in degree with the concentration used. This is followed by

(2) A gradual re-expansion, lasting several minutes. This may go on to

(3) Further swelling, with the exudation of 'bubbles' from the cell. These bubbles can be made to swell or shrink by varying the osmotic pressure of the medium. The swelling of the cell is very marked with concentrations of formalin below 7 %. The addition of saline reduces the degree of swelling but only to a minor extent. The final appearance of the cell, even after bubbling has occurred, does not always greatly deviate from normal. Nevertheless, the cell has undergone considerable structural and chemical alterations. It is not certain how far these results apply to blocks of tissue in which mechanical conditions may prevent gross swelling and bubbling. It seems probable, however, that the same underlying changes will occur.

Dr BOURNE remarked that in view of the fact that it had been claimed that there is a decrease in the cytoplasmic contents of cells under different physiological conditions it would be interesting to know if any of these passed out into the exuded bubbles described.

Professors YOFFEY and YOUNG inquired if the authors had made any measurements of cells fixed in formalin or formalin vapour with and without saline. Dr CRAWFORD replied that while they had made no precise measurements it could be said that the presence of saline produced less swelling. He considered that increased concentration of saline would most likely produce less shrinkage.

Dr R. BARER read a paper on 'The reflecting microscope in anatomical research'.

The reflecting microscope which has been in use at Oxford for over a year is one constructed by Dr C. R. Burch, F.R.S. It consists of a reflecting condenser and a reflecting objective, each composed of one large concave mirror and a small convex mirror. The mirrors are made from speculum metal, the large mirrors being aspherical in order to correct for spherical aberration. In its simplest form the microscope has a numerical aperture of 0·65, but this can be increased to 0·98. The great advantage of the reflecting microscope is that it is completely achromatic. Once the object is focused in light of *any* colour the instrument is

in focus for *all* wave-lengths, from the far ultra-violet to the infra-red. This is particularly valuable for ultra-violet photography and especially for ultra-violet absorption spectroscopy of cellular constituents. Such work has hitherto been carried out using a quartz refracting microscope (Caspersson), a technique which necessitates the use of monochromatic light, and which is very laborious. With the reflecting microscope an image of the cell under study is projected across the slit of a spectrograph and a photograph is taken. If a line source (e.g. a mercury arc) is used, an image of that part of the cell lying across the slit is obtained *simultaneously* in all wave-lengths emitted by the source. The use of a continuous source of radiation, such as a hydrogen arc, enables a complete absorption curve to be obtained automatically by means of a recording microphotometer. Examples of the applications of these methods to the study of the structure and cyto-chemistry of various types of cell are discussed.

Another feature of unusual interest is the long working distance of the reflecting microscope. In the present model the distance between the lowest part of the objective and the object is 13 mm. This enables micromanipulation to be performed under magnifications of 600–700 times. The surfaces of organs *in situ* are now available for microdissection. High-power capillary and skin microscopy can also be carried out.

Professor YOFFEY inquired if the author had studied erythroblasts of marrow, Dr BARER replied that he was actually working on this problem and on foetal blood cells.

Professor J. D. BOYD read a paper on 'Argentophil cells in foetal ectodermal epithelia'.

During observations on the development and nerve supply of the human external auditory meatus, dendritic cells, with a marked affinity for silver, were observed in the epithelial plug. These cells possess the morphological characteristics of microglia and of the resting phase of cells of the reticulo-endothelial system. They are believed to represent foetal stages of the dendritic cells (Langerhans' cells) of the adult skin. Such cells have also been found in numerous other epithelia of human and other mammalian material at the author's disposal. In the present communication their distribution in the cutaneous epithelia of the head region is described and their origin and possible significance discussed.

Dr BAXTER inquired if argentophil cells had been noted in epithelial cords not of ectodermal origin, such as the vaginal canal, and in which breakdown to form a lumen took place. Professor BOYD replied that this problem was being studied.

Dr BACSICH remarked that the cells described by Professor BOYD are known as the cells Langerhans to the dermatologists, who believe that they are either part of the macrophage system of migrating leucocytes. He had tried in vain to find an elective staining method for these cells. In Professor BOYD's specimens, as a by-product of a silver impregnation for neuro-histological purposes, these cells are represented with unsurpassed clarity, so that a successful approach to the study of their physiological and pathological behaviour is now possible.

To a question by Dr WEDDELL, Professor BOYD replied that these dendritic cells stain with methylene blue.

Drs P. BACSICH and G. M. WYBURN read a paper entitled 'Subcutaneous homo- and heterografts of the umbilical cord'.

Recent experiments (Bacsich & Wyburn, *Proc. Roy. Soc. Edinb.* **62**, 1947) suggest that the sulphurated mucoproteins of cartilage and the cornea are responsible not only for the avascular nutrition and translucency (or transparency), but also for the constant success of these tissues as homografts.

Theoretical considerations led us to assume that the non-sulphurated and consequently more easily hydrolysable mucoproteins of the umbilical cord ground substance would allow survival only for a limited period by affording a somewhat reduced protection to the graft.

Results of present experiments on the fate of subcutaneous homo- and heterografts of umbilical cords of guinea-pigs seem to bear out this assumption.

The significance of these findings and the bearings of these results on skin homografts are discussed.

Dr G. CAUSEY read a paper on 'The effect of pressure on nerve-fibre size'.

This work was undertaken to see if it is possible to show a centrifugal pressure along the axon of a myelinated nerve fibre.

Circumferential pressure round the nerve to the medial head of the gastrocnemius of the rabbit was obtained by placing the nerve across a glass chamber filled with mercury, and sealed with a gel of sodium alginate at the openings through which the nerve was passed. The nerve was then fixed and sectioned. Measurements were made, on photographs of the sections, of the area of the whole nerve bundle and of the myelinated fibres.

Comparisons of sections above, at and below the site of pressure and of a control nerve from the opposite limb showed that for pressure of 80 mm. Hg and over there was (*a*) a mean decrease of 25 % in the area of the nerve bundle at the site of pressure, (*b*) a decrease in the mean fibre diameter at the site of pressure, (*c*) an increase in the mean fibre diameter above and below the site of pressure, the increase above being significantly less than the increase below the site of pressure. This difference is in agreement with the existence of a centrifugal pressure along the axon.

In reply to Dr BOWDEN who inquired about the effect of blood vascular occlusion on the nerve fibres Dr CAUSEY replied that ligature of the artery and vein together did not block conduction, but application of pressure after such ligature would block conduction and alter fibre size.

Dr ROMANES asked if the thickness of the myelin sheath could be taken as a reliable guide, to which Dr CAUSEY replied that estimates of myelin thickness, apart from the total diameter, had not been made.

Dr J. R. HINSHAW (introduced by Dr G. WEDDELL) read a paper on 'The pattern of innervation in the iris'.

The innervation of whole preparations of iris from white rabbits has been studied by the aid of the intravenous methylene-blue technique (Feindel, W. H., Sinclair, D. C. & Weddell, G., *Brain*, 1947, **70**, 495). Many marginal nerve trunks anastomose to form several concentric plexuses from which are derived networks of fine beaded fibres. These networks innervate both the internal and external surfaces and are found in relation to the circular muscle. Perivascular nerves are rarely seen in the iris and this is in contrast to the prominence of perivascular nerves observed in many other tissues prepared by this method. The interstitial cells are well stained and show no connexion with the nerve fibres.

The iris is an ideal structure for the experimental study of autonomic innervation and the normal pattern of innervation has been mapped out in detail as a basis for such an investigation.

Dr MEYLING commented that Dr HINSHAW's statement that the interstitial cells showed no connexion with the nerve fibres, could be due to a partial staining of the nervous structures. He had often observed this in his own preparations, but when these were well and 'totally' stained a connexion with post-ganglionic fibres could always be made out. The nervous networks of beaded fibres demonstrated by Dr Hinshaw are probably the processes of anastomosing interstitial cells of which the nuclei are not stained. In reply Dr HINSHAW stated that he was sure that there was no connexion between the interstitial cells he had demonstrated and the nervous network.

Drs G. WEDDELL, W. H. FEINDEL and D. C. SINCLAIR read a paper on 'Pain sensibility in deep somatic structures'.

The hypothesis of Lewis (1938, Suggestions relating to the study of somatic pain, *Brit. med. J.* **1**, 321) that the skin and the deeper somatic tissues are supplied by two separate systems of pain nerves has been examined in the light of the recent work of Weddell, Sinclair & Feindel (1948, An anatomical basis for alterations in the quality of pain sensibility, *J. Neurophysiol.* (in the Press)), who found that alterations in the quality of pain sensibility can be produced by alterations in the pattern of the pain-producing impulses reaching the central nervous system. A preliminary examination of the innervation of

certain deep somatic structures has been made, and it has been found that this innervation differs both in pattern and in density from that of the skin. These histological findings are sufficient to explain the observed differences in the subjective and objective results of painful stimulation of the skin and of deeper lying structures respectively. There is thus no need to postulate two separate pain-conducting systems supplying somatic tissues.

Dr MEYLING remarked that the terminal nervous structures demonstrated are probably autonomic nerve fibres, because they exhibited the typical varicosities which are always seen in peripheral parts of the autonomic nervous system.

Drs E. J. FIELD and J. B. BRIERLEY read a paper on 'The anatomy of the spinal nerve root lymphatic drainage'.

That a functional communication exists between the spinal subarachnoid space and the prevertebral lymph nodes in the rabbit has been demonstrated in a previous communication to the Society. The precise course of the lymphatic vessels concerned could not, however, be determined by the method then adopted to show this connexion. It has now been possible by a method of retrograde filling of these lymphatics from the abdomen after ligation of the thoracic duct in the thorax, to demonstrate their course in full. Vessels which commence in the substance of the posterior spinal nerves pass ventrally and receive tributaries from the region of the nerve roots and from the interior of the vertebral body. Such lymphatics end in one of several longitudinal channels on the front of the spine. Along such channels are the small lymph nodes which constitute the 'regional' nodes of the spinal subarachnoid space. These vessels merit attention in any consideration of the route whereby infective agencies may extend from the lymphatic to the central nervous system, particularly as they do not possess valves. Their existence, moreover, may afford a plausible explanation of some of the puzzling results in recent experimental work on poliomyelitis.

Dr BOURNE inquired why the authors assumed that the carbon particles passed through the cytoplasm of the cells—it seemed more likely that they passed between the cells. Dr FIELD replied that various authors had claimed that the pathway was through the cytoplasm and not intercellularly, and they concurred with this view.

Professor BOYD remarked that there were Hyrtl preparations demonstrating lymphatics in relation to the meningeal sheaths of dorsal root ganglia in the museum of University College, London.

Dr P. GLEES read a paper on 'The localization of efferents from Area 4 in the internal capsule and corpus callosum of the macaque'.

Following an experimental study of the distribution of the cortico-spinal fibres from Area 4, further experiments have been performed to study the localization of the cortico-spinal and other descending fibres from Area 4 in the internal capsule. There is, however, no definitive area within the internal capsule assigned to a particular subdivision of Area 4. On the contrary, the fibres from all subdivisions of Area 4 intermingle in their descending course. The callosal distribution of Area 4 and its subdivisions have been investigated as well.

Professor BOYD commented on the limitations of the pyramidal system in some animals, to which Dr GLEES replied that in those animals which have a small pyramidal tract, often ending at the cervical level, a similar comparatively small sensory cortical projection occurs, as had been shown recently by Adrian for the pig where only impulses from the snout region reach cortical level.

Professor YOUNG inquired about recovery rates following lesions, and as to whether there was any evidence that leg areas 'take over' from arm areas. Dr GLEES replied that as far as the monkey is concerned, fairly quick motor recovery results after small lesions in Area 4. He assumed that neighbouring parts of the motor area can 'take over'—e.g. the leg for the arm area. Certain anatomical arrangements favour this view, but more experimental evidence is required.

Dr J. T. AITKEN read a paper entitled 'Effect of peripheral connexions on the maturation of regenerating nerves'.

Previous work has shown that the type of connexion made at the periphery by a regenerating nerve influences the degree of maturation. Experiments were performed in which a muscle nerve was allowed to regenerate along different distances during a 100-day period. It was found that the greater the distance traversed by the regenerating fibres, the larger they became. Motor fibres which had travelled a long distance in an adjacent sensory nerve became larger than those travelling a short distance. The effect was most marked where the sensory nerve was still connected to its peripheral end-organ system.

Other regenerating nerves were implanted into muscles, some already innervated and others denervated, and the degree of maturation assessed after a 100-day period. The nerve implanted in the paralysed muscle produced a much more completely regenerated nerve than did that of normal muscle on the other side. The implants into normal muscle, however, were more mature than those nerves ending blindly as a neuroma in the fascia or where the regenerating tract leads to the peripheral end organs of a sensory nerve.

It appears, therefore, that the process of maturation during regeneration is influenced by such factors as the distance the young fibres have to travel, and also the possibility of making functional motor endings on muscle fibres. Further experiments showed that fibres turn back at a neuroma; if the latter is removed, the number of fibres in the stretch of nerve above it decreases. The crowding produced by the recurrent fibre diminishes the size of all fibres in the nerve.

Dr ROMANES asked whether the turning back of the fibres at the neuroma would not of itself lengthen the pathway of regeneration, and Dr AITKEN pointed out that any such effect would be limited to the very small-sized fibres.

Dr BOWDEN stated that the organization of voluntary movement was related to the size of the nerve fibres, and commented on the effect of length as an added factor affecting the fibre diameter. On inquiry by Dr GLEES it was stated that the paralysed muscles responded to a smaller stimulation of the implanted nerve than did the normal muscle.

JUNE 1948

The Summer Meeting of the Society, for the Session 1947–48, was held in the Department of Anatomy, of the University of Bristol, on Friday and Saturday, 25 and 26 June 1948, the President (Professor A. B. APPLETON) in the Chair.

Drs J. B. BRIERLEY and E. J. FIELD and Professor J. M. YOFFEY read a paper entitled 'Passage of indian ink particles from the cranial subarachnoid space'.

It has been generally believed that particulate matter cannot, under normal pressure conditions, escape from the cranial subarachnoid space to the extracranial lymphatics. In experiments on rabbits, using a particle suspension of known size, ink was introduced into the cisterna magna, replacing an equivalent quantity of cerebrospinal fluid at a pressure of 120 mm. of water. Within a few hours ink was found in the submucous lymphatics of the nose, having travelled along the subepineural space of olfactory nerve bundles and along intraneural clefts not lined by endothelium.

Dr R. J. HARRISON inquired if macrophages were present in the normal mucous membrane. Dr FIELD replied that some indian ink particles were within phagocytic cells while others were free. The main emphasis, however, was not on the segregation of indian ink particles by macrophages but on its presence in pathways leading from the cranial subarachnoid space.

Drs J. B. BRIERLEY and E. J. FIELD read a paper on 'The retro-orbital tissues as a site of functional connexion between the subarachnoid space and the lymphatic system'.

The well-known extension of the subarachnoid space around the optic nerve has been further investigated in the rabbit by the indian ink technique described in a previous

communication to the Society. Ink particles were seen to penetrate the optic-nerve sheath throughout its whole extent and coronal sections showed that their concentration decreased from the nerve towards the bony wall. The retractor bulbi muscle was heavily stained with ink, the particles lying in parallel columns between their fibres. The recti muscles were less intensively stained, but in some cases the ink tracked forwards to their scleral insertion. No true lymphatics were ever observed within the orbital cavity.

The diffuse spread of particles appeared to justify the injection of ink behind the globe in order to define the further course of fluid drainage. Twenty-four hours after injection ink was noted in the upper pole of the deep cervical lymph nodes. There was dense accumulation of ink around the nerves entering the orbital apex.

The suggestion is advanced that the cerebrospinal fluid contributes to the tissue fluid of the orbit, draining normally towards the apex of that cavity, where it is absorbed into lymphatic vessels which are afferents of the deep cervical lymph nodes.

Thus the retro-orbital tissues around the optic nerve cul-de-sac may stand in the same relation to the cervical lymphatic system as the epidural tissue of the spinal nerve roots to the pre-vertebral lymph nodes.

Dr WHILLIS commented on the relative size of the spaces around the optic and olfactory nerves and Dr BRIERLEY said that he could not offer any explanation for this difference.

To Professor KIRK who inquired about the relationship of Tenon's capsule to the subarachnoid space, Dr BRIERLEY replied that this space was very difficult to define in the rabbit and that the ink introduced was confined to the posterior surface of the bulb.

The PRESIDENT in thanking the authors said that he was pleased to see the expansion of the work in the lymphatic system begun by Professor Yoffey. The present findings, he thought, may prove to be significant in connexion with the mechanism of exophthalmic goitre.

Drs W. H. FEINDEL and A. C. ALLISON read a paper on 'Intravital methylene blue as a method for staining degenerating fibres in the central nervous system'.

An intravenous technique which permits a much wider application of methylene blue as a vital stain for nervous tissue (Feindel, Sinclair & Weddell, *Brain*, **70**, 495, 1947) has been modified (Feindel & Allison, *Science*, 23 April 1948) to allow for a study of degenerating fibres in the brain. In rabbits perfused with methylene blue 5 days after enucleation of one eye, degenerating axons in the optic tract are rendered remarkably conspicuous by their intense and metachromic staining as compared with normal fibres. In addition, morphological changes of early degeneration, such as fibrillation and vesiculation of the axis cylinder, are clearly demonstrated. The results are briefly described and compared with those obtained by the Marchi method and by metallic silver impregnation. It is likely that the method may find useful application for defining accurately the anatomical distribution of fibre degeneration produced by selective experimental lesions of the central nervous system.

Professor GOLDBY inquired if the technique had been applied to the peripheral nervous system. Dr FEINDEL replied that the method was not yet perfected, but it might well be extended to the peripheral nervous system. He further remarked that the results were rather empirical and that further work on the chemistry of the technique was in progress.

Mr RICHARDSON inquired if oxidation products might be liberated when this dyestuff was used, and was informed that further research on the chemistry of the technique together with spectroscopic investigations might elucidate this problem.

Dr H. A. MEYLING (introduced by Professor R. D. LOCKHART) read a paper entitled 'Structure and significance of the peripheral autonomic nervous sytem'.

To study the peripheral autonomic system both silver impregnation and vital methylene-blue staining are used. Schabadasch's modification of the latter, specially valuable, demands very exact use. Dilute methylene blue (0·01 %), buffered (pH 5–6) with $MgBr_2$ (1·25–2·0 g/l.) and glucose (2·0 g/l.), is either injected intra-arterially or the tissue is immersed in the solution for 10–20 min. The tissues are then left for 10–20 min. in the solution without

methylene blue, fixed in 2·11 % ammonium iodide in saturated ammonium picrate and mounted in glycerine saturated with ammonium picrate. Precise timing and exact buffering, differing for each tissue, will stain totally the peripheral extension of the autonomic nervous system, while other tissues, especially connective tissues, are unstained. In all the tissues examined (conducting system of the heart, endocardium and pericardium, stomach, intestines, urinary bladder, gall bladder, small blood vessels, carotid sinus and aorta in the horse, cow, sheep and rat) a nervous network was found, consisting of autonomic interstitial cells of Cajal. As the post-ganglionic and efferent fibres are connected with this network, since Nissl substance is present in the vicinity of the nuclei, and since the processes of the interstitial cells show typical varicosities and, especially in silver preparations, a neurofibrillar structure, it is concluded that these cells are of nervous nature.

Most authors agree that autonomic fibres end as a network, containing nuclei. Since these nuclei are surrounded by a network of neurofibrils and these neurofibrils also form a fine network in the processes of the cells, these cannot be Schwann cells. Sympathetic and parasympathetic fibres are unlikely to form one network, which, persisting after the post-ganglionic and afferent fibres are cut, must be formed by the processes of the interstitial cells. In the different tissues this network differs only in the form of the pattern and the number of nuclei. An anatomical conception of a primitive intrinsic nervous network (similar to the sensory-motor network in invertebrates), upon which end both the post-ganglionic and afferent fibres, harmonizes with the physiology, explaining continuing local function after autonomic section and providing a basis for the view that adrenalin acts at the periphery upon some intermediary between post-ganglionic terminations and tissue cells.

Professor Boyd said that he considered the author's technical methods insufficient to permit accurate recognition of interstitial cells. He was doubtful if the plexus formation of interstitial cells of Cajal did in fact exist. Dr Meyling replied that he had no doubt about the plexus ramification as a continuous network. He had employed dilute solutions of methylene blue, well buffered, and which could only give a selective staining.

Dr D. E. Bolt and Professor J. M. Yoffey read a paper on 'Inability to detect macrophages in degenerating nerves'.

In a number of rats the sciatic nerve was divided and the animals subsequently killed at intervals ranging from 1 to 73 days. Although the cut ends were left free, in the experiments of longer duration some regeneration occurred. Animals were deeply stained intravitam with trypan blue for 5 days before they were killed. No dye-containing cells were ever found in the interior of a nerve bundle, though macrophages in the epineurium were heavily stained.

To Dr Sanders, who inquired if there was any evidence of conversion of Schwann cells into macrophages as alleged by some authors, Professor Yoffey replied that staining of Schwann cells by trypan blue was an infrequent occurrence—when they did so the nuclei showed quite a deep stain also.

Mr Richardson suggested that owing to the interference with blood vessels of the nerve no dye got through, and consequently the macrophages, though they were there in abundance, could not be demonstrated simply because the dye did not reach them. Professor Yoffey replied that there was ample evidence that the dye did get through and they hoped to present this evidence later. If anything, the permeability of the blood vessels in degenerating nerves was increased.

Dr E. L. Patterson read a paper on 'The arterial blood supply of the sympathetic trunks in man'.

A macroscopic survey of the arteries concerned in the blood supply of the sympathetic trunks has been made by injection of the arterial system of foetuses with indian ink. Ganglionic branches from the ascending pharyngeal and inferior thyroid arteries to the cervical trunks, from the superior intercostal, aortic intercostal and lumbar arteries to the thoracic and lumbar trunks, and from the lateral and middle sacral arteries to the sacral trunks, are described.

Professor KIRK congratulated the author on the painstaking and delicate dissection required to reveal the blood supply to these sites. He was surprised that the vertebral artery did not give a rich blood supply to the inferior cervical ganglion.

Dr MORTON inquired if there were any differences in the pattern of the blood supply at different age periods. Dr PATTERSON replied that the specimens dissected were all at or near full-term and that he had observed no significant differences in them.

Dr FEINDEL asked if the arteries to the sympathetic trunk were arranged in segmental fashion. Dr PATTERSON replied that in the thoracic and lumbar regions the arrangement was in general segmental, each ganglion receiving one or more branches from the corresponding segmental artery, but that not infrequently the ganglion received additional vessels from adjacent segmental arteries above or below its level.

Dr F. K. SANDERS read a paper on 'The fate of nerve homografts in the rabbit'.

Pieces of tibial or peroneal nerve 2–10 cm. long were taken from donor rabbits and used to fill gaps in the peroneal nerves of other rabbits, being fixed in place with clots of autogenous plasma. Grafts were removed and examined histologically at intervals from 2 days to 6 months after operation, their appearance being compared with that of control autografts placed on the opposite side of the same animal.

The Schwann cells of an autograft survive and multiply, and the myelin breaks up and is removed, so that in the later stages autografts are indistinguishable from normal peripheral stumps. The Schwann cells of homografts, however, undergo complete destruction within the first week after grafting, the graft at the same time being invaded by large numbers of lymphocytes, these being especially numerous in the region of the junctions. The collagenous framework of the graft, however, persists for a long time, being invaded by large numbers of macrophages, presumably to remove the debris of degenerated nerve fibres and destroyed Schwann cells. Regenerating nerve fibres, accompanied by Schwann cells, are able to invade such grafts from the central end. At the same time, Schwann cells from the host nerve migrate into the graft from its distal end, and when the graft is short (less than 3 cm.) the two streams of cells meet somewhere within the graft, giving such grafts at a late stage an appearance which does not differ strikingly from that of autografts. In long grafts (5 cm. and over), owing probably to a failure of vascularization, the two streams of cells fail to meet. As a result such grafts at a late stage show a proximal zone resembling a successfully reinnervated peripheral stump, separated from a distal zone resembling a degenerated but uninnervated distal stump by a dense collagenous plug.

The implication of these and other findings with reference to the mechanism of destruction of homografts was discussed.

To a question by Professor YOFFEY as to whether or not there was evidence of haematogenous migration of macrophages once vascularization had occurred, Dr SANDERS replied in the affirmative.

Dr E. P. SAMUEL read a paper on 'The innervation of the articular capsule of the knee joint'.

A detailed histological study of the innervation of the articular capsule of man and of the cat has been undertaken to provide an accurate anatomical basis for a further inquiry into the sensitivity of these structures. It was found that a large proportion of the medullated and non-medullated nerves which ramified in the joint capsule did so in company with the blood vessels. They were distributed in a plexiform manner through the fibrous capsular ligament and synovial membrane, and gave off branches which (*a*) innervated the blood vessels; (*b*) formed a true nerve plexus in the synovial membrane (this plexus received branches from the nerves which entered the joint tissues independently of the blood vessels); and (*c*) ended in various types of free nerve endings.

Some new light has been thrown on the nature of the specialized endings in the joint capsule. All the types of endings previously described have been seen while examining 200 feline and 20 human knee joints, and it is felt that previous differences of opinion regarding their existence have arisen because some former investigators examined too

small a series of specimens. The exact nature of the nerve fibres in the articular capsule has hitherto remained unsolved, and in an effort to acquire more precise knowledge the knee joints of twelve sympathectomized cats have been studied and compared with joints of normal animals. The conclusions reached were that, though a large component of the nerves in the joint capsule were of a sympathetic origin, the synovial membrane, contrary to the views of Gerneck (1932), Plasserman and Daubenspeck (1938), and others had a substantial somatic nerve supply.

Dr AITKEN remarked that careful histological technique must be employed to distinguish nerve fibres; unless continuity from larger fibres to smaller ones can be traced, then the results must remain inconclusive. Dr SAMUEL replied that he was convinced that his technique demonstrated nerve fibres only.

Drs M. REISS, F. E. BADRICK and J. M. HALKERSTON (introduced by Professor J. M. YOFFEY) read a paper on 'The uptake of radioactive tracers by the pineal gland'.

In confirmation of Borell and Örstrom it was found that the pineal takes up per mg. fresh weight considerably more P^{32} than any other organ of the body. Borell and Örstrom are inclined to ascribe, therefore, a special hormonal function to the pineal in phosphorus metabolism. This assumption was found to be incorrect, since also the iodine uptake (I^{131}) of the pineal is, with the exception of the thyroid, considerably higher than all the other organs.

It has, on the other hand, been found that P^{32} and I^{131} uptake of the pineal is still further increased after hypophysectomy. Some antagonistic action between pituitary and pineal concerning the uptake of radioactive tracers is therefore assumed.

Dr J. HAMILTON asked whether the calcareous deposits in the pineal gland had any relationship to phosphorus uptake. Dr REISS replied that he did not know, but that this problem would be worth while investigating.

Dr JACOBY commented on the observed increase in cell content of the pineal following hypophysectomy, and inquired if there was any evidence of mitotic activity. Dr FIELD, replying for the authors, said that no mitosis had been observed, but that this need not be regarded as unusual; some rapidly growing embryonic tissues showed none.

Dr F. B. ROBINSON and Professor J. M. YOFFEY read a paper entitled 'Effects of cold and adrenaline on the suprarenal cortex of the adult male Wistar rat'.

Fifty adult male rats were exposed to cold at 0° C. for times varying from 5 min. to 7 days. Forty-four rats were given single intraperitoneal injections of adrenaline and killed at times varying from 5 min. to 1 week afterwards. Six rats were given repeated injections of adrenaline three times a day for times ranging from 1 to 7 days. Studied by means of the Schultz and other histochemical reactions, the suprarenal cortex showed depletion commencing within 5–10 min. after exposure or injection. In general, there is a close similarity in the response of the cortex to adrenaline and cold.

Dr REISS remarked that the interesting changes reported in the cholesterol distribution in the adrenal might be due to the endogenous mobilization of corticotrophic hormone in the cold. It has been shown that the corticotrophic hormone considerably reduces the cholesterol content of the adrenals in a few hours after injection; the cholesterol content being restored or even increased 2–3 hr. afterwards. The reduction in cholesterol is probably connected with the increased cortin production which takes place when rats are kept in the cold. He thought it most interesting that the changes described by Dr Robinson occurred mainly in the zona fasciculata and reticularis, while the zona glomerulosa remained unaltered. This may be in agreement with the assumption that the production of corticosterone (responsible for carbohydrate metabolism, etc.) takes place in the zona fasciculata, which also atrophies after hypophysectomy, while the production of desoxycorticosterone (responsible only for water and salt metabolism) in the zona glomerulosa is apparently not influenced by the pituitary anterior lobe.

Dr J. D. Green read a paper on 'The comparative anatomy of the vessels and nerves of the pituitary body'.

The blood supply and innervation of the hypophysis has been studied in thirty-eight representatives of existing classes of vertebrate. With some reservations for Agnatha and Chondrichthyes, pending further study, the following generalizations appear to hold:

(1) The derivatives of Rathke's pocket obtain their blood supply from the vascular mesenchyme, which at first separates them from the diencephalon.

(2) The venous effluent passes caudally and laterally into the vascular plexus of the saccus endolymphaticus in amphibians or into the cavernous sinus in higher forms.

(3) A hypophysio-portal circulation is developed in *Salientia*, is constant in higher forms and is characterized by a secondary vascularization of the median eminence of the tuber cinereum (found also in some caudata) and by collection of the effluent blood into portal channels which then supply the adenohypophysis.

(4) In *Necturus* the dorso-caudal part of the infundibulum receives an independent blood supply. Progressive enlargement and specialization of this region, apparently paralleling the first acquirement of a land habitat, leads to the neural lobe of higher forms.

(5) The histology of the neural lobe is suggestive of direct secretion from nerve terminals rather than through the pituicytes.

(6) Endings are often seen in the pars intermedia. Vasomotor-like endings are frequent in the median eminence and rather rare in the pars tuberalis, but never in the pars distalis are nervous structures seen, though reticulum may closely simulate them.

Dr Walls suggested that a radio-opaque substance such as thorotrast might be employed to demonstrate the course of the circulation. This would provide a permanent photographic record of the direction of the blood flow. Dr Green replied that the direction of blood flow had been observed directly by Houssay *et al.*, and by himself in a variety of Amphibia. He agreed that it was desirable to show this in higher vertebrates, but he doubted whether the method suggested was as reliable as actual observation of the living circulation.

Dr G. W. Harris asked why the author stated that the median eminence of the neurohypophysis contained relatively few nerve fibres as compared with the infundibular process. Dr Green replied that the fibres which passed through the median eminence branched when they reached the infundibular process, giving the appearance of more nerve fibres in the latter.

Dr G. W. Harris read a paper on 'Ovulation in the rabbit'.

A technique has been developed for stimulating the basal areas of the brain in a group of conscious rabbits simultaneously and for prolonged periods. By this method it has been found that stimulation of the tuber cinereum in the unanaesthetized rabbit, for as short a period as 3 min., may be followed by a full ovulatory response. Direct stimulation of the anterior pituitary, pars intermedia or pituitary stalk, for periods up to 7½ hr. was not followed by ovulation. These results are discussed in relation to the possibility of neurovascular transmission of stimuli from the hypothalamus to the anterior lobe of the pituitary via the hypophysial portal vessels.

Dr Jacoby inquired if the author had any idea of the nature of the stimuli passing along the blood vessels. Dr Harris replied that there was some evidence for acetylcholine as a possible hormonal transmitter of the stimuli down the hypophyseal portal vessels, though the work of Taubenhaus and Soskin, on which this hypothesis is based, was performed on the pseudopregnancy response in rats, and could be questioned on that ground. There was other evidence put forward by Markee, Sawyer and Hollingshead that some adrenaline-like substance might be concerned, for these workers injected adrenaline directly into the pituitary gland in rabbits and obtained ovulation. But this evidence is also open to doubt, for the injection of any substance directly into the pituitary will result in the destruction of some glandular tissue, and possibly in the liberation of stored hormone into the blood stream.

Dr J. S. Baxter read a paper on 'Alkaline phosphatase activity in the renal tubules'.

In the post-natal kidney of the rat, alkaline phosphatase is present only in those proximal

convoluted tubules which have previously been shown (Baxter & Yoffey—in the Press) to be functional as judged by their ability to store trypan blue. The enzyme is absent, or present in negligible traces, in the mesonephros of this form. It appears in the proximal segments of the metanephric tubules relatively late in pre-natal life and its activity is not very great when compared with the kidney after birth.

In the sheep, on the other hand, enzyme activity is marked in the mesonephros at the earliest stages examined (7·8 mm.), and continues until well after the time when it is first seen in metanephric tubules (29 mm.). Its activity in the latter is well marked during the remainder of pre-natal life.

Since the presence of the enzyme probably denotes a functional maturity of the mesonephric and metanephric tubules, the differences between the results in the rat and the sheep are probably to be correlated with the type of placentation in each.

Alkaline phosphatase is also present in the 'secretory' segment of the pronephric tubule in the tadpole stage of the frog and in fishes shortly after hatching. It would appear, then, to be an important substance in the vertebrate nephron.

Professor HAMILTON inquired concerning the state of affairs before the link up of secretory and collecting tubules during development. Dr BAXTER replied that alkaline phosphatase did not appear as a cytoplasmic enzyme until the tubules became structurally and functionally differentiated.

Professor J. D. BOYD read a paper on 'Argentaffin dendritic cells in urogenital epithelium of male mammalian embryos'.

Silver-impregnated material of rabbit, pig and human embryos and foetuses shows the presence of an extensive system of dendritic cells in the epithelium of the prostatic and penile portions of the urethra, in the epithelial fusion between the prepuce and the glans penis and in related ducts. The dendritic cells are similar in morphology to those found in embryonic epithelium of the head and to the dendritic cells ('melanoblasts') of post-natal skin. Their distribution and possible significance are discussed.

Professor YOFFEY inquired if the processes of these cells actually enter the substance of neighbouring cells. Professor BOYD replied in the negative and added that in skin, cells of similar character 'pay off' melanin into epithelial cells.

Dr C. C. MACKLIN's communication entitled 'Mapping the epithelium of the finer bronchioles by supravital silverization' was read by Professor W. J. HAMILTON.

The fresh collapsed lungs of normal adult white mice were filled with ammoniacal silver solution, quickly drained and at once refilled with 10% neutral formalin. Strips of terminal bronchiolar epithelium, seen *en face* in flattened cleared frozen sections, were mottled, with irregular rows or islands of dark golden brown intermingled with clear tracts of analogous disposition. These sharply contrasting areas marked the air-presenting faces of two distinct and well-differentiated cell types composing the bronchiolar epithelium, respectively the smaller silverized or dark, and the larger unsilverized, light or clear cells.

The faces of the dark cells were made up mainly of fine, brown, silverized granules of uniform size and distribution, and their clear-cut borders were featured by points, joined by dark silvered lines, often concave outwardly. Towards the alveolated air-ducts these faces became narrow, relatively inconspicuous, and insunken between the protruding clear cells. In lateral views afforded by unstained paraffin sections the silverized cells relatively remote from the alveoli were quadrangular, with the faces underlying the cilia appearing as rodded lines of golden brown in which two layers of granules could sometimes be discerned; while the incurved side walls and flat bases were represented by sheets of fine black granules. Distally these cells frequently appeared as sharply limned truncated pyramids with flaring bases and narrow brown apical surfaces at the bottoms of funnel-like depressions. They may be represented on the alveolar walls by the epicytes.

The clear cells, everywhere unciliated, formed islands and rows interwinding among the silverized cells. Peripherally they increased in collective area relatively to the dark cells.

Each bore on its dome-like air-surface a variable rounded plaque of silverized granules. Distally these cells became lower, broader and less bulging, and gave way abruptly to the 'bare areas' of the alveolar walls.

Dr A. d'A. Bellairs read a paper entitled 'Observations on the snout of *Varanus*'.

Some aspects of the anatomy of the snout in this genus of lizards are described, with special reference to the nasal capsule, nasal and vomeronasal organs and lachrymal apparatus. The morphology of these structures differs in certain respects from that of other lizards; a feature of special interest is the presence of two separate lachrymal ducts on either side. The long anterior duct discharges into the duct of the organ of Jacobson, as in snakes; the short posterior duct opens farther back into the nasal cavity.

Certain resemblances between conditions in *Varanus* and those in snakes are described as follows: the configuration of the nasal organ and its capsule, with a long anterior chamber and cupola, the posterior situation of Jacobson's organ, the absence of choanal grooves, the long protusible tongue and the course and relations of the anterior lachrymal duct. In the orbital region, however, the chondrocranium of *Varanus* is characteristically saurian, with a high interorbital septum and planum supraseptale, contrasting with the platytrabic condition of snakes. The bearing of these findings on the systematic position of *Varanus* and the evolution of snakes is discussed.

Professor Boyd inquired about the functional significance of the two separate lachrymal ducts in *Varanus*, to which Dr Bellairs replied that this seemed obscure.

Professor Yoffey asked how the reconstructions were made and was informed that this was done by the method of graphic reconstruction on squared paper. To a question on the cribriform plate by the same speaker Dr Bellairs replied that there was no cribriform plate in reptiles. The olfactory bulbs project over the sphenethmoid commissures and the olfactory nerve fibres pass up through the fenestra olfactoria.

Dr Harper commented on the large size of the lateral recess in *Varanus* and inquired about its functional significance. Dr Bellairs replied that although present in some other lizards it was very well developed in *Varanus monitor*, though not in some other species of the genus. Its large size was perhaps associated with the elongation of the anterior chamber of the nasal sac, but its functional significance was unknown.

Professor R. D. Lockhart showed a film of a case of 'Voluntary eversion of the eyelids' and discussed the mechanism involved.

This accomplishment (or affliction) was shown by a medical student, and the main interest of this film lay in the mechanism of execution. Probably the movement can be acquired by practice. Cases have been recorded where the individual has been able to evert the eyelids on one or other side voluntarily.

As a rule the exponents, including the present case, prefer to fix the lateral parts of their orbicularis oculi by pressing their hands against the temples and pushing backwards, but occasionally the movement is executed without this assistance.

This film was taken by Dr W. Clark Souter, Ophthalmologist, Aberdeen, who thought that anatomists might be interested in devising an explanation of the action.

Professor Whitnall suggested that the levator palpebrae superioris and probably also the frontalis played a large part in the mechanism of voluntary eversion of the upper eyelid. Professor Lockhart disagreed, and stated that the eversion of the tarsal plate which he thought was smaller than normal in the present case was probably effected before the lid was opened. He considered that the peripheral fibres of the orbicularis oculi were alone responsible for the movement.

Dr Panting said that he thought the levator palpebrae superioris contracted before the orbicularis oculi and thus initiated the movement of the tarsal plate.

In thanking Professor Lockhart for the interesting film demonstration the President suggested that the presence of anomalous muscle fibres in the lateral part of the orbicularis oculi might help in the explanation.

Mr F. R. WILDE read a paper on 'The nerve supply of the iliac vessels in man'.

The general pattern of innervation of the arteries to the extremities is well known. The precise origin and number of such nerves, their ultimate source, and the degree of individual variation still await clarification. Moreover, the nerve supply of veins, regarding which anatomical literature is scanty, has assumed clinical importance of recent years.

The present communication summarizes the conclusions reached from dissection of the vascular nerves that supply the common and external iliac vessels, in full-term human foetuses.

Dr N. TERESCHENKO expressed admiration of Mr Wilde's skill as a dissector, and remarked that he had worked with a French surgeon who considered that periarterial sympathectomy was invariably successful. In the case of the femoral artery special attention to two points was necessary, viz.: (1) that the first inch of the profunda must be carefully stripped as well as the femoral artery itself, as the nerve fibres following the profunda later returned to the main femoral trunk; and (2) that the nerve fibres travel in the superficial layer of the intima as well as in the adventitia and must be destroyed to ensure success; accordingly the denuded parts of the arteries should be painted with a carbolic solution which can be washed away after a few minutes.

Dr F. W. FYFE presented a communication entitled 'Some features of the interlocking pattern at the upper epiphysial surface of the femur with reference to "slipped epiphysis"'.

The interlocking of elevations and depressions on the opposed metaphyseal surfaces of the head and neck of the femur is not mentioned in the masterly paper of Key (1926), where the opinions of 102 authors are discussed. Nor is it cited in available papers from 1926 to the present, the most recent anatomical experiment reported being by Ollerenshaw and Wood Jones, whose attempts to force off the epiphysis in two normal femora aged 14 and 15 years, removed post mortem, caused fracture distally in the neck.

Key emphasizes the strength in children of the neck periosteum, which in adolescence atrophies towards the adult type. The epiphyseal junction is a curved surface, like the spherical head, but flatter centrally. If the periosteum were not tightly attached, angular stresses would tend to dislodge the neck from the cupped head.

The upper end of the femoral neck of a boy, whose epiphysis looks normal but had been loosened by bomb blast, shows a tendency for antero-posterior slipping after bisection coronally, periosteal support thus being reduced. This tendency is due to ridges running antero-posteriorly. Other femora show the elevations (papillae and ridges) in a variety of patterns. One type noted has the ridges running from a flat or depressed centre to the periphery, an arrangement capable of opposing stresses from all directions. Another type has a more definite arrangement, with antero-posterior ridges at anterior and/or posterior quadrants. All have areas with papillae scattered indiscriminately, mostly in the medial and lateral quadrants (the condition throughout the flat, horizontal epiphysis of the elephant), but these papillae are often low and rounded in contour. This series is at present too small for categorical statements, but since it is improbable that early enough cases will ever be examined at biopsy or post-mortem, it may help to explain the clinical fact that trivial injuries, harmless if sustained as stresses in some directions, are, nevertheless, the only etiological factors in 30 % of cases (endocrine imbalance being quoted as the cause in the rest); and if the shearing stress is sustained in an antero-posterior plane by a femur whose epiphyseal ridges run in a similar direction, the head may be the more easily dislodged, other factors being equal, e.g. periosteal strength and cartilage-to-bone cohesion.

Professor MITCHELL said that the existence of papilliform elevations and reciprocal depressions interlocking like those of dermis and epidermis, was recognized by many orthopaedists as a stabilizing mechanism at epiphyseal junctions. Dr Fyfe's suggestion that these might be definite papillary epiphyseal patterns was novel and interesting, and it was conceivable that certain patterns might prove less efficient in resisting either shearing or angular stresses and so favour diastasis. If further investigations confirmed this suggestion, the matter would be of considerable interest to orthopaedic surgeons.

Professor YOFFEY suggested that a similar study might be made at other sites, e.g. at the

iliac crest, and Dr WHILLIS remarked that microscopic examinations of these regions should be made.

Professor C. M. WEST read a paper entitled 'Human remains from Wookey Hole'.

Professor WEST gave an account of the topography of the Wookey Hole Caves and showed specimens of skulls and limb bones which had been recovered, during recent diving operations, from the River Axe in its course through the caves. He had studied the remains of ten skulls, and he showed two further skulls which had just been recovered. The skulls were of both long and round types with cranial indices from 80·2 to 70·7. There were large, small and intermediate varieties, the largest with a capacity of 1740 c.c. and the smallest 1260 c.c. Some of the skulls recalled the Cromagnon type, others the Neolithic type of Busk. All were characterized by a marked projection of the occipital squama. One of the skulls showed evidence of an interparietal suture below the occipital bulge. Romano-British pottery had been found close to some of the skulls. Associated femora and tibiae showed platymeria and platycnemia, and gave a stature of from 5 ft. to 5 ft. 6 in. A remarkable maxilla with a dental arcade of Roman arch type and of great width, comparable with the St Brelade maxilla, was also shown.

Mr E. J. MASON (introduced by Professor WEST), who is in charge of the Cave Diving Group concerned with the operations during which the bones and pottery were discovered, gave further details of the sites where the remains were found.

Dr H. HUGHES inquired as to how the remains came to be under water. Mr MASON replied that there was no evidence that the water had risen or that the ground had subsided. One might speculate that the remains had been thrown into the water, but at this stage it would be better to await the results of further investigations.

Dr W. R. M. MORTON read a paper entitled 'A pre-villous human conceptus of about 10 days of age'.

A specimen of an early human conceptus removed by curettage from a woman suffering from menorrhagia and abdominal pain is described. The ovum is incomplete, and lacks an embryonic mass, and is possibly pathological. The trophoblast is differentiated into cyto- and syncytio-trophoblast, and the trophoblastic vesicle is lined by extra-embryonic mesoderm. There is no zone of necrosis in the surrounding endometrial tissue, and the uterine glands are free from extravasated blood or pent-up secretion. The menstrual and copulation histories and the state of development of the ovum indicate its age as approximately 10 days. The position of the specimen relative to previously described human ova of similar development is discussed.

Professor BOYD remarked on the shallowness of the implantation, and said that the presence of the coarse fibrinous coagulum suggested a pathological change.

Professor WEST said that he thought the ovum showed signs of abnormality in the poor development of the syncytiotrophoblast, in the size of the chorionic vesicle compared with its depth of implantation, and in the condition of the lacunae.

Professor HAMILTON remarked that it was now just over 40 years since Bryce and Teacher described an early ovum. That embryo is now recognized as pathological. If, however, Dr Morton had discovered his embryo shortly after that of Bryce and Teacher it would have confirmed the appearances seen in their embryo. In his opinion Dr Morton's embryo was either pathological or had been dead for some time. As far as he could judge from the photomicrographs it seemed as if it were being expelled from the endometrium. The group of cells seen towards the periphery of the ovum might be degenerating cells of the inner cell mass. The general appearance of the syncytiotrophoblast resembled that seen in the embryo no. ER 785 of the Hertig/Rock series.

In his reply Dr MORTON said that he thought that maternal blood cells were leaking into the lacuna; vessels followed in serial section do communicate slightly with lacunae. While the endometrium appeared to be normal, he admitted that there may have been an endocrine imbalance somewhere to produce the effect described.

Dr L. Harrison Matthews (introduced by Dr J. S. Baxter) read a paper on 'The uterine mucosa in Balaenopterid whales'.

Specimens of the uterine mucosa from thirty-four antarctic whales have been examined; data on the state of the ovaries, pregnancy if present, and mammary glands were available for correlation with the conditions found in the mucosa. The changes found show the course of formation of the maternal part of the placenta from the stage of progestational proliferation to the end of pregnancy, and the involution of the mucosa after parturition.

After ovulation there is a great increase in congestion in the superficial capillaries of the mucosa. The network of capillaries becomes so close that in middle and late pregnancy the entire surface of the mucosa is in effect covered with a film of blood. No extravasation was found at any stage. The epithelium becomes reduced to an extremely thin layer of cells. After ovulation, crypts also are formed as depressions in the surface of the mucosa: as pregnancy advances the stratum compactum becomes increasingly invaded by them. This cutting up of the surface of the mucosa, together with the proliferation of the tissue intervening between the crypts, gives the mucosa a spongy texture, which appears in sections as a large number of much-branched processes. These arise from the trabeculae between the crypts, and project into the uterine lumen with a dendritic outline, reaching an extreme degree of complexity during later pregnancy. Gland ducts open at the bottom of some of the crypts, and the capillary network extends over the surface of the dendritic processes.

The distribution of the crypts appears to be more widespread than in the Odontoceti, and are little if at all smaller in the amniochorionic than in the allantochorionic region. The stratum spongiosum is relatively thicker and richer in glands whose wide lumina, holding a coagulum during later pregnancy, favour the view that an embryotrophic material is produced and secreted, probably into spaces between chorion and mucosa at the bottoms of the crypts.